Ecotoxicology of Antimicrobial Pharmaceutical and Personal Care Products in Illinois Rivers and Streams

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Acknowledgements

Funding

- Illinois Sustainable Technology Center

Collaborators

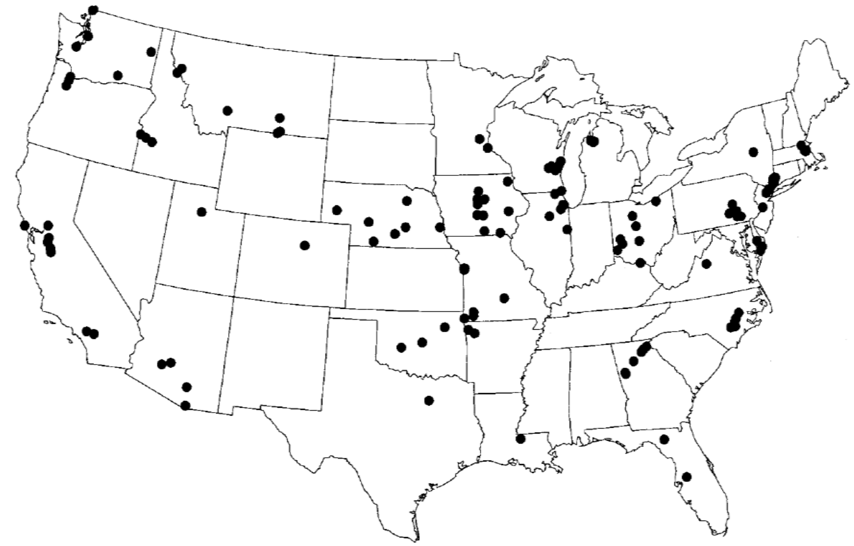
- Emma Rosi-Marshall
  - The Cary Institute of Ecosystem Studies
- John Scott
  - Illinois Sustainable Technology Center
- Teresa Chow
  - Illinois Sustainable Technology Center
- Monte Wilcoxon
  - Illinois Sustainable Technology Center

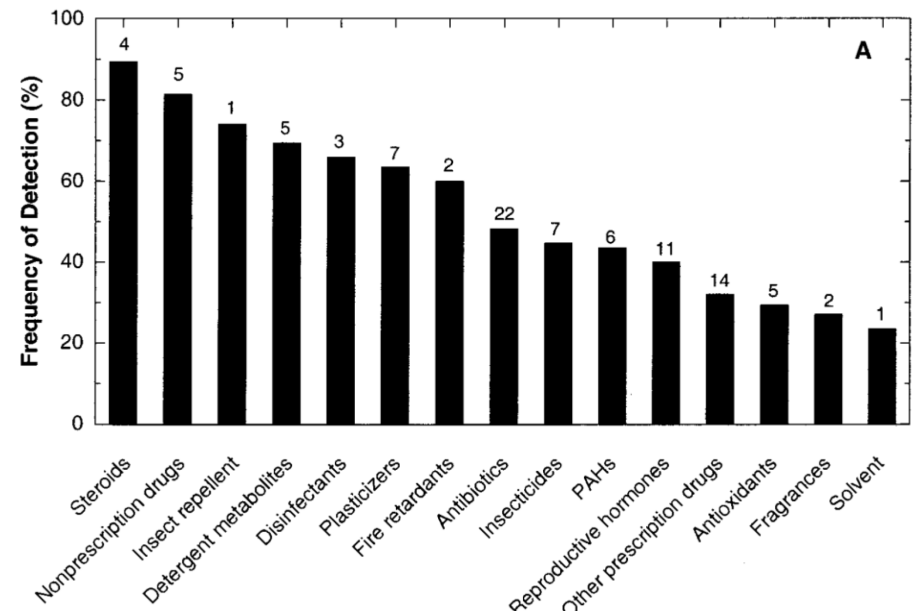
Growing Concern about Pharmaceuticals and Personal Care Products in Surface Waters

- PPCPs: Prescription and over-the-counter drugs, antibiotics, disinfectants, soaps, detergents, cosmetics, etc.
- Many of these products are used due to their specific biological effects
- A growing number of these compounds have been detected in freshwater environments

2000 USGS Survey

- Surveyed 139 Streams in the United States
  - Selected streams susceptible to contamination
- Tested water for 95 contaminants
  - Contaminants found in >80% of streams
- The most commonly detected compounds were steroids, insect repellant, caffeine, **triclosan**, fire retardant, detergent metabolites

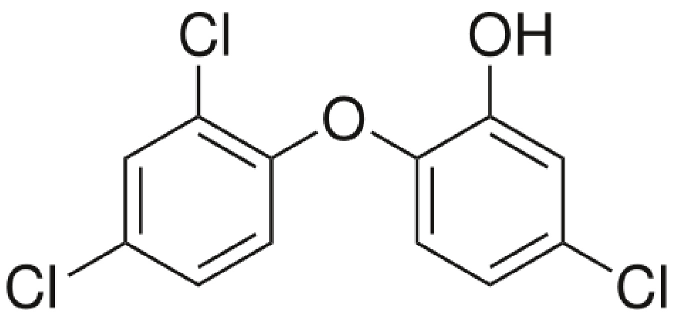




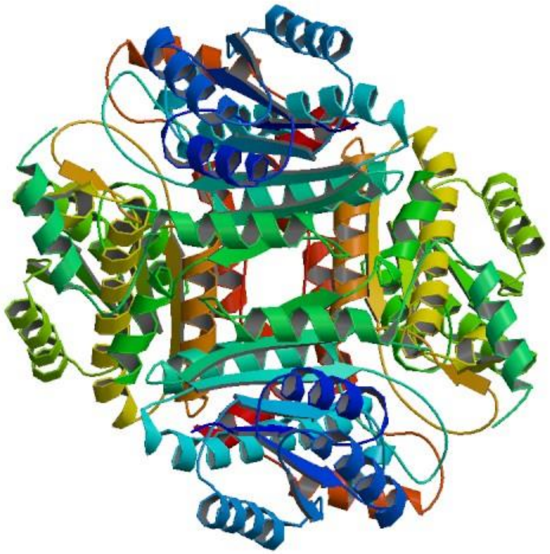
Kolpin *et al*., 2002. Environ. Sci. Technol.

Antimicrobial: Triclosan

- Synthetic broad spectrum antibacterial compound
- Irreversibly binds to the enoyl-acyl carrier protein reductase
  - An essential enzyme in the bacterial fatty acid biosynthetic pathway (McMurry *et al.*, 1998)
- Discovered in 1970s
  - Used mainly as a disinfectant in hospitals until 1990s
- Currently found in more than 700 consumer products
  - Soaps, detergents, toothpaste, cleansers, plastics, textiles, etc.



2,4,4’-trichloro-2’-hydroxydipheyl ether



Enoyl-acyl Carrier Protein Reductase

Sources of Triclosan

- Triclosan will enter domestic wastewater through normal use
  - Triclosan has been detected in domestic wastewater (McAvoy *et al.*, 2002; Bester, 2003; Kanda *et al.*, 2003)
- Several studies have examined the fate of triclosan in WWTPs
  - WWTPs effectively removed ~ 95% of triclosan from wastewater (~79% was degraded and ~15% sorbed to sludge) (Kanda *et al.*, 2003; Sabaliunas *et al.*, 2003)
- Since removal is not 100%
  - **We hypothesized** that WWTPs could be sources of small but continuous amounts of triclosan to streams

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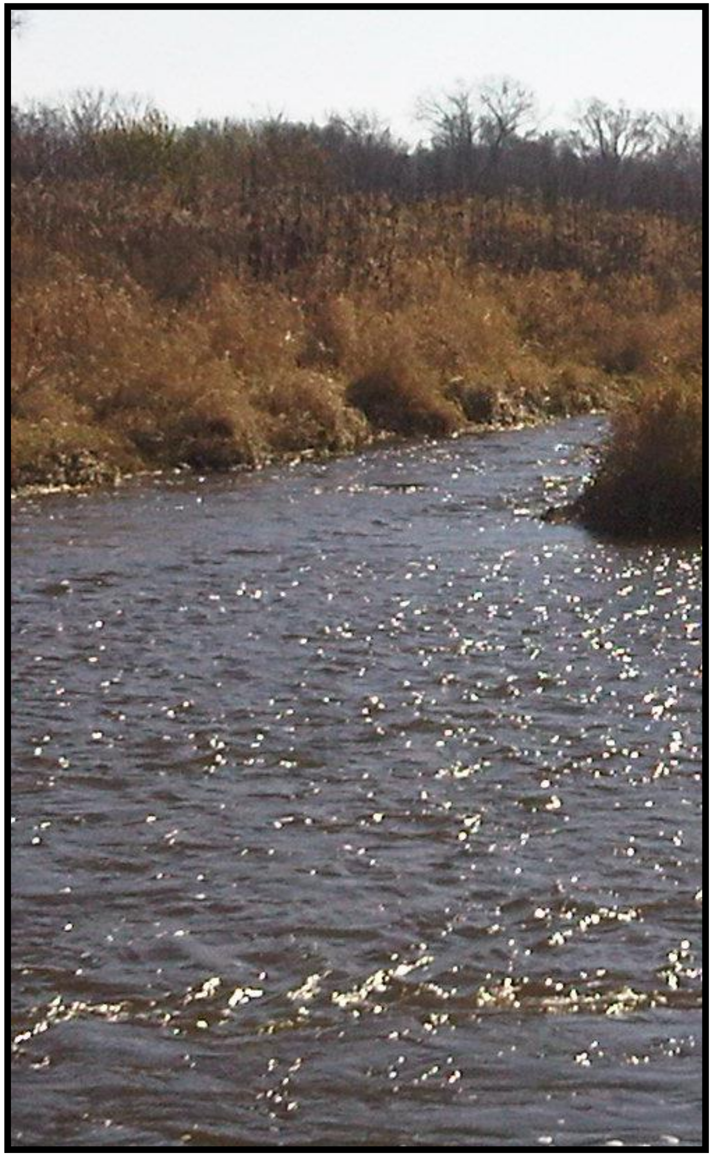
Fate of Triclosan

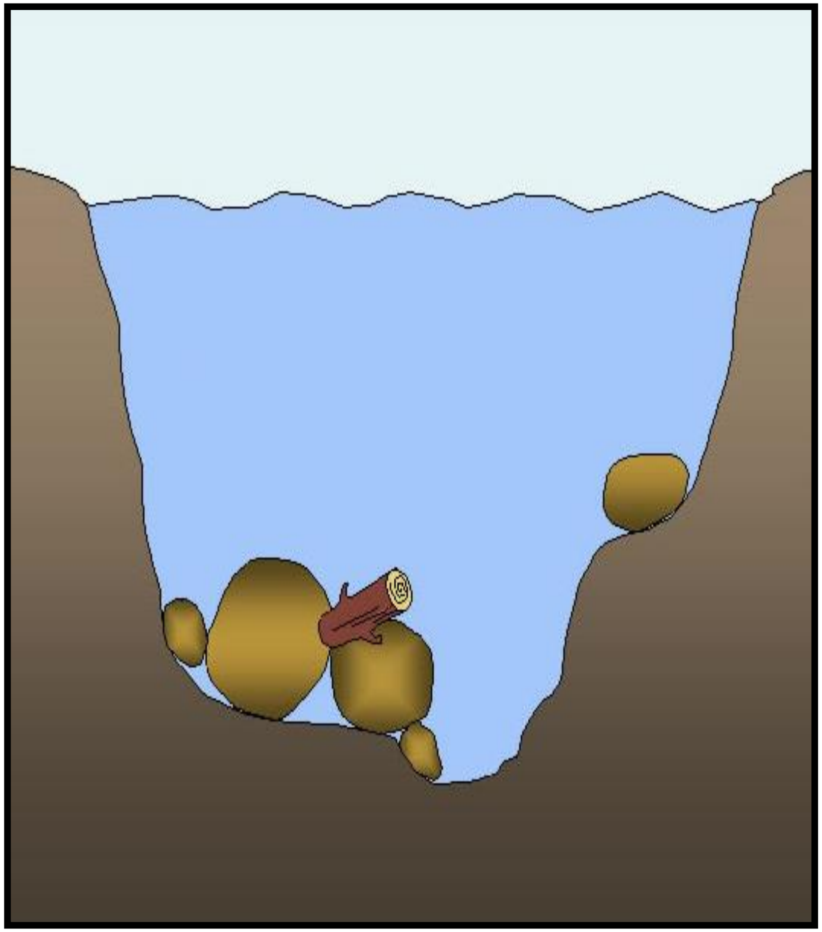
- Most studies have measured triclosan in the water of aquatic habitats
  - Kolpin et al., 2002
- Triclosan is hydrophobic with low aqueous solubility
  - Should partition to sediments
- Triclosan seems to be resistant to degradation in sediments
  - Has been detected in 30 year old sediments (Singer *et al.*, 2002)
- **We hypothesized** that triclosan would accumulate in stream sediments and could affect benthic bacterial communities



Benthic Microbial Communities

These communities are important components of stream ecosystems

- Bacterial numbers are generally much higher in freshwater sediment than in the overlying water (Sander and Kalff 1993)
- Benthic microbial communities contribute to ecosystem processes
  - Primary production
  - Nutrient cycling
  - Decomposition of organic material
  - Bioremediation of pollutants
- Primary production (GPP) in benthic microbial communities drives whole-stream nutrient uptake (Tank et al 2008)



http://individual.utoronto.ca/michaeliwama

Effects of Triclosan on Bacteria

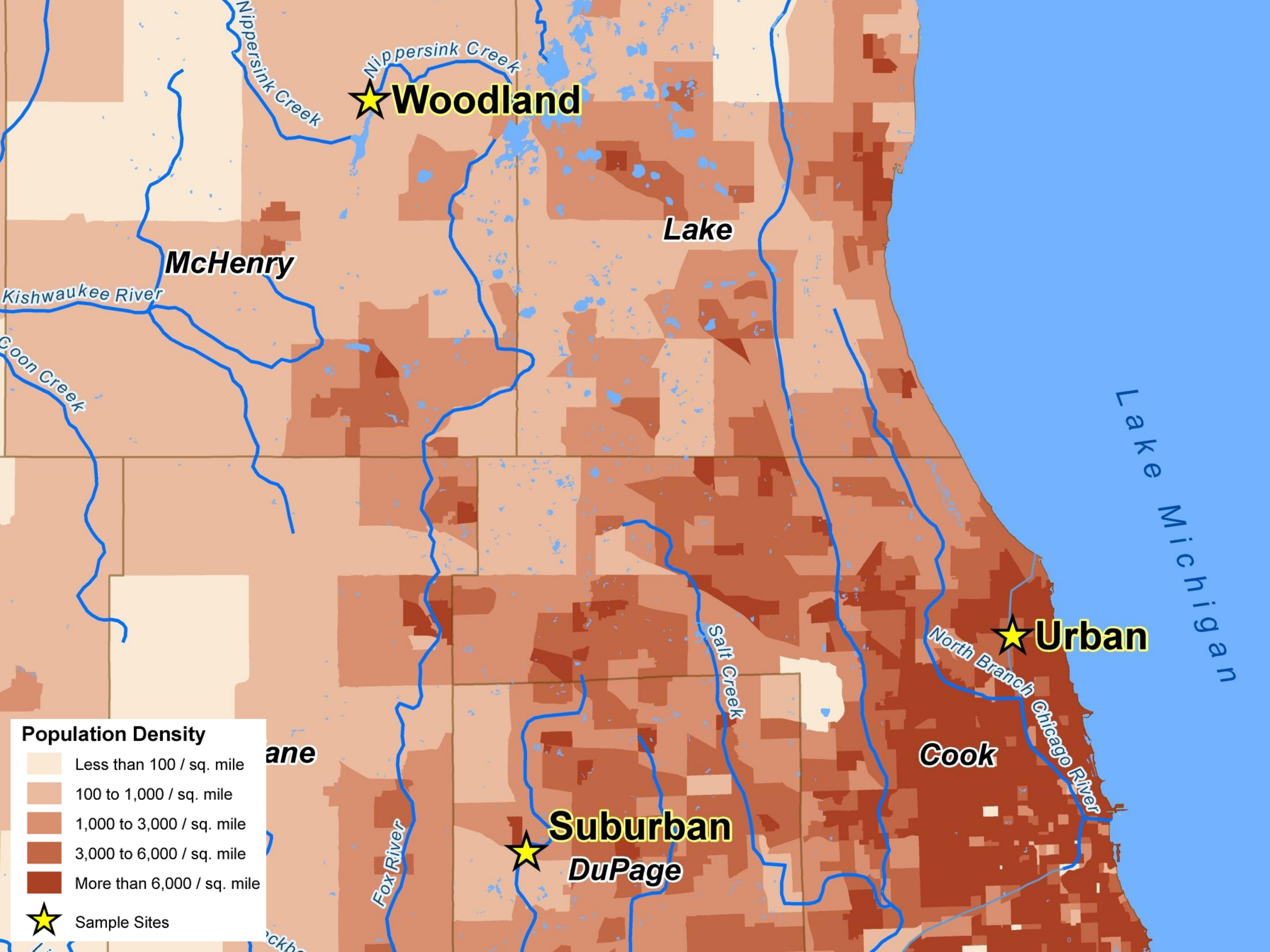
- Triclosan inhibits bacterial growth
  - Binding to the enoyl-acyl carrier protein reductase
- Triclosan resistant bacteria have been developed in the laboratory
  - Mutations in *fabI* (Heath *et al*., 1998)
  - Overexpression of *fabI* (McMurray *et al*., 1998)
  - Efflux pumps (Chuanchuen *et al*., 2003)
- Link between triclosan resistance and resistance to other antibiotics
  - Chuanchuen *et al*., 2001; Braoudaki and Hilton, 2004

Research Questions and Hypotheses

- **Question 1:** Are WWTPs point sources for the entry of small but continuous amounts of triclosan into sediments of lotic ecosystems?
  - **Hypothesis 1:** Concentrations of triclosan in the sediments downstream of WWTPs will be significantly higher than those found upstream
- **Question 2:** If we find triclosan in sediments, is it significantly affecting on sediment bacterial communities?
  - **Hypothesis 2:** Triclosan will have a negative effect on bacterial abundance
  - **Hypothesis 3:** Triclosan exposure will select for more resistant bacterial communities
  - **Hypothesis 4:** Triclosan will alter the taxonomic composition of sediment bacterial communities

Experimental Design

- Collect sediment samples from streams that receive effluent from WWTPs
  - **Urban Site:** North Shore Channel, Chicago, IL
  - **Suburban Site:** West Branch DuPage River, DuPage County, IL
- Collect sediment samples from a stream with very low human impact to serve as a control
  - **Woodland Site:** Nippersink Creek, McHenry County, IL



Woodland
Nippersink Creek
Nippersink Creek
Lake
McHenry
Kishwaukee River
Coon Creek
Lake Michigan
Urban
North Branch Chicago River
Salt Creek
Cook
Suburban
DuPage
Fox River
Kane
Population Density
Less than 100 / sq. mile
100 to 1,000 / sq. mile
1,000 to 3,000 / sq. mile
3,000 to 6,000 / sq. mile
More than 6,000 / sq. mile
Sample Sites

Sampling Site Characteristics

Physical Characteristics and Land Use

Site	Width (m) *	Discharge (cfs) **	Watershed (sq. mi)	Watershed Land Use (%)				
				Residential	Commercial / Industrial	Agricultural	Forest / Open	Vacant
Urban	30	28.5	25	63	17	0	10	0
Suburban	23	51.3	127	33	4	17	11	17
Woodland	11	161	12.3	21	1	49	10	10

Drury et al., In Prep

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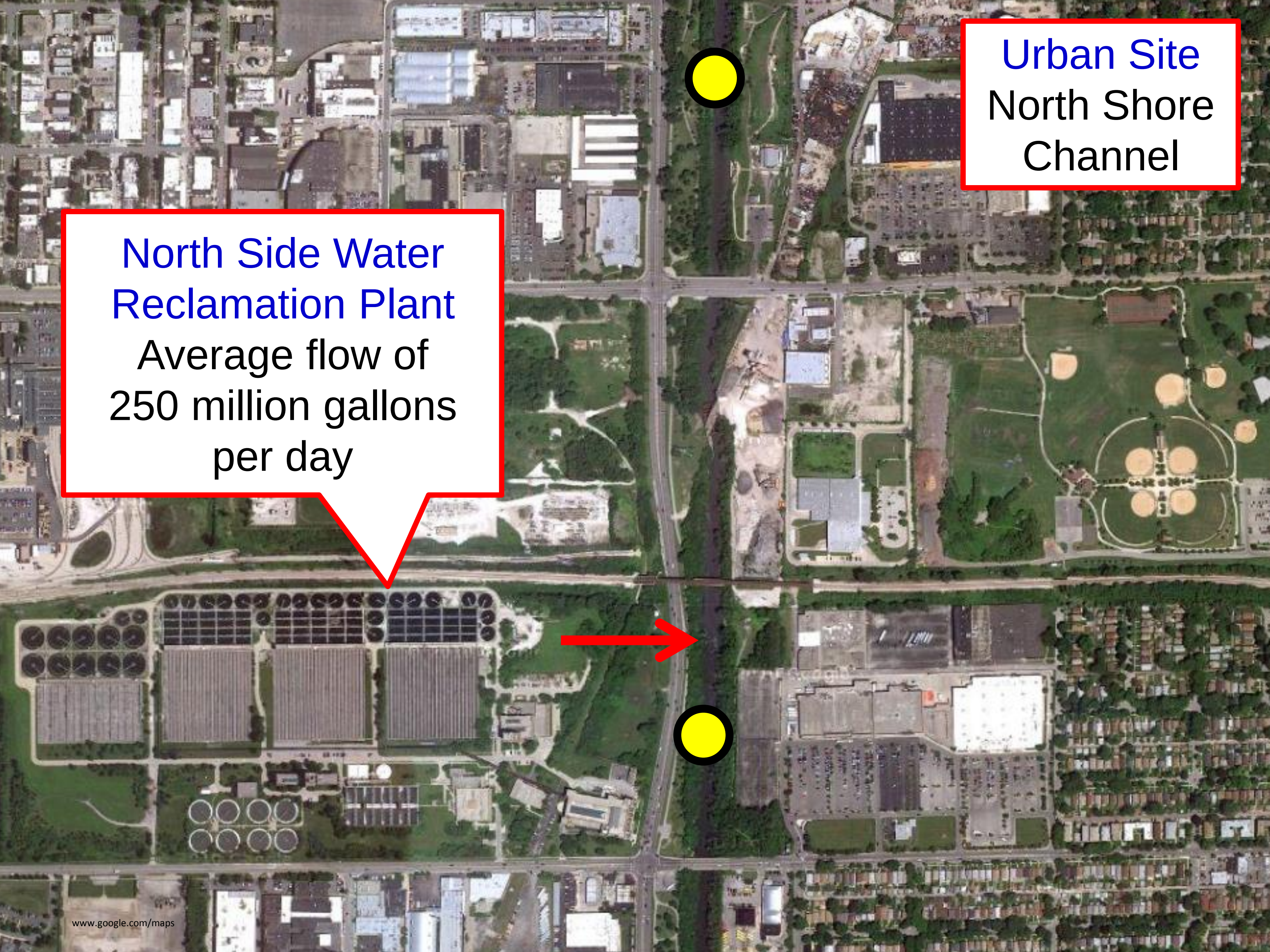
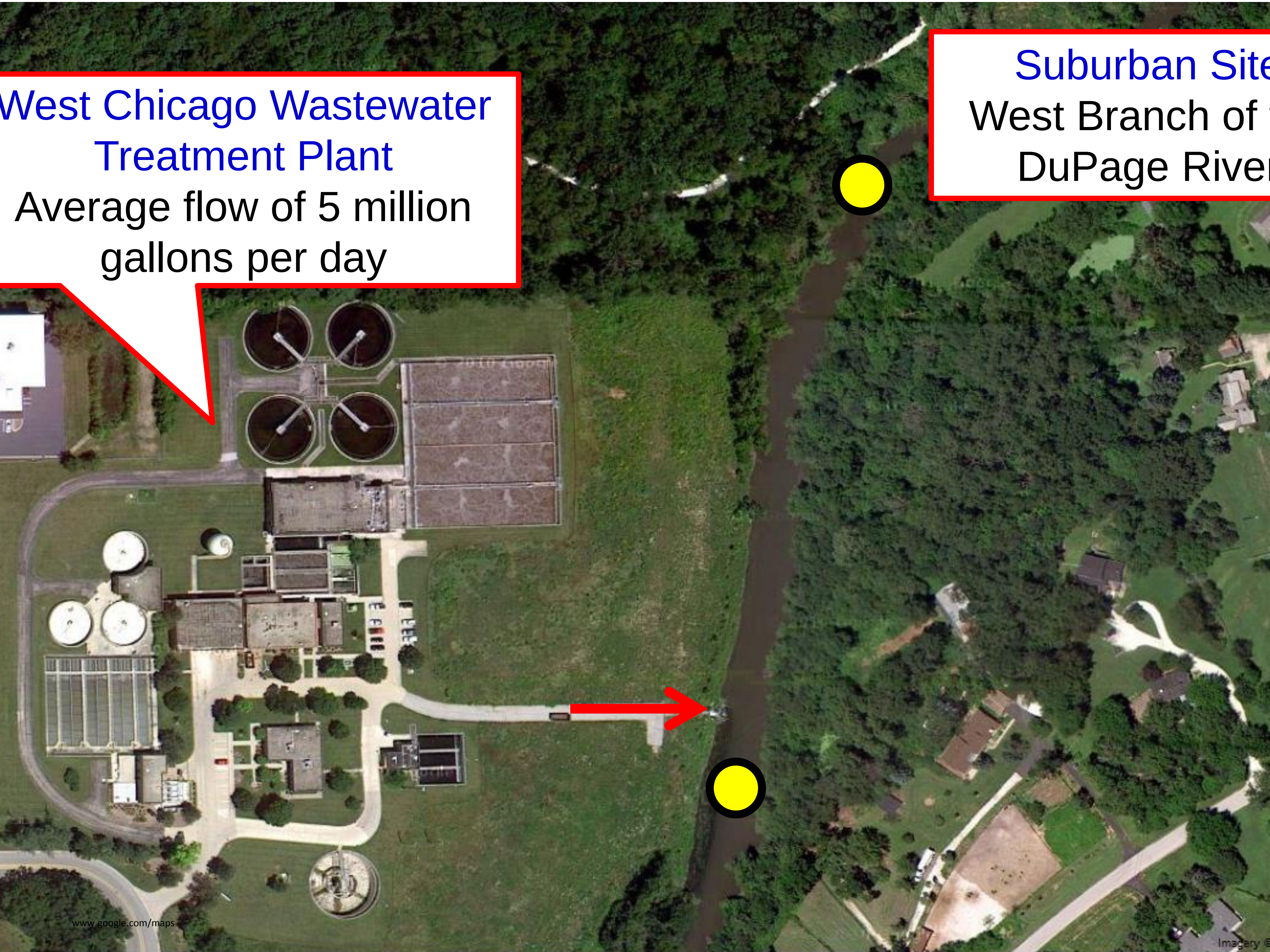
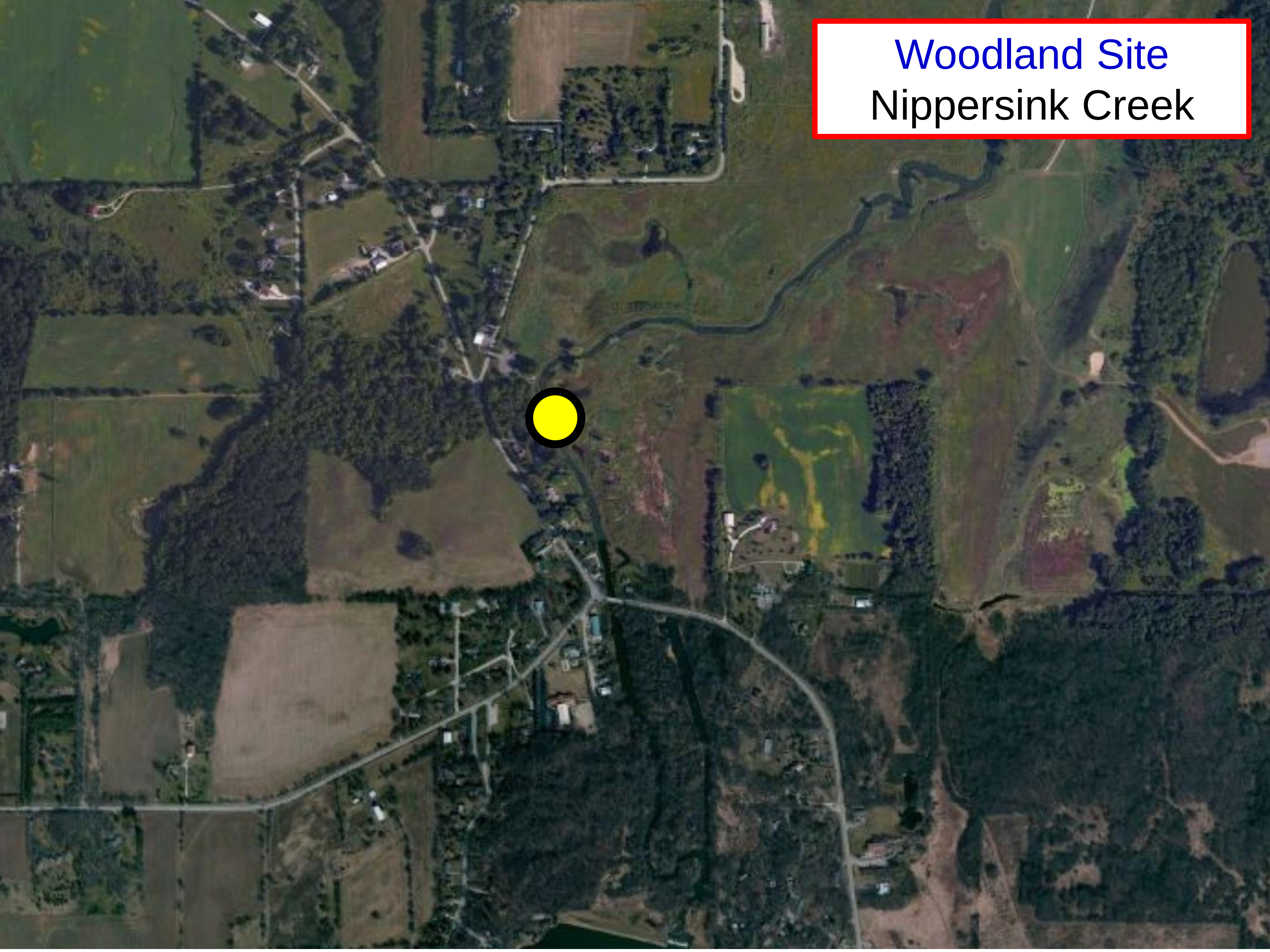


Urban Site
North Shore Channel
North Side Water Reclamation Plant
Average flow of 250 million gallons per day
www.google.com/maps



West Chicago Wastewater Treatment Plant
Average flow of 5 million gallons per day
Suburban Sit
West Branch of
DuPage Rive
www.google.com/maps

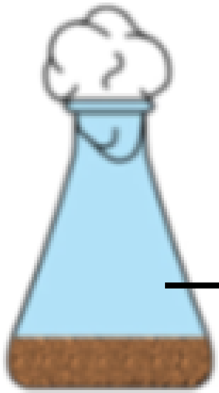

Woodland Site
Nippersink Creek

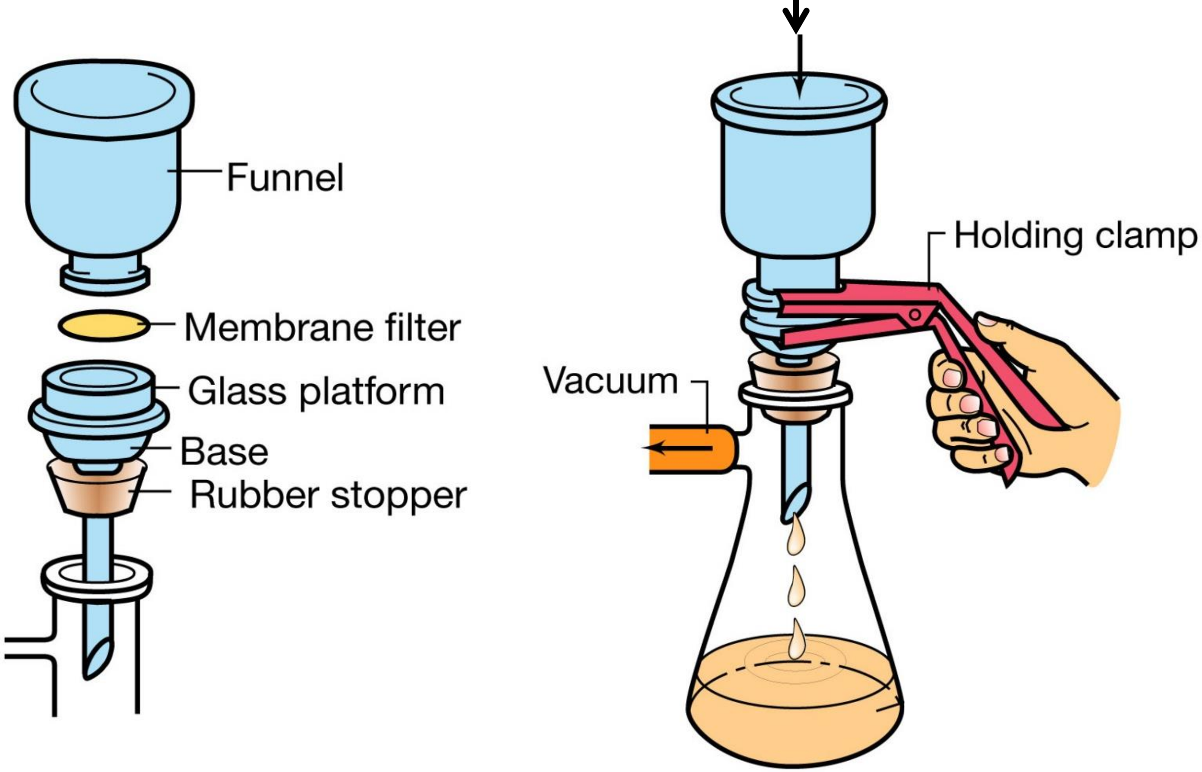
Experimental Design

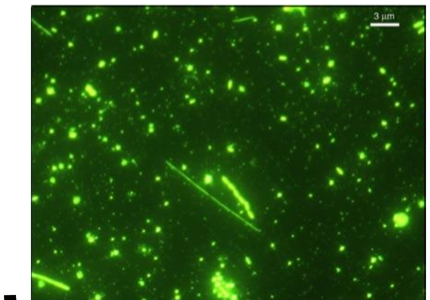
- Five sampling sites
  - Urban Upstream, Urban Downstream
  - Suburban Upstream, Suburban Downstream
  - Woodland
- At each site we collected
  - 5 replicate water samples
  - 5 replicate sediment samples from each location
- Measured triclosan concentrations in sediments
  - Accelerated solvent extraction followed by MS/MS
- Profiled sediment bacterial communities
  - Bacterial abundance
  - Community triclosan resistance
  - Bacterial community composition

Bacterial Abundance

Direct Epifluorescence Counts



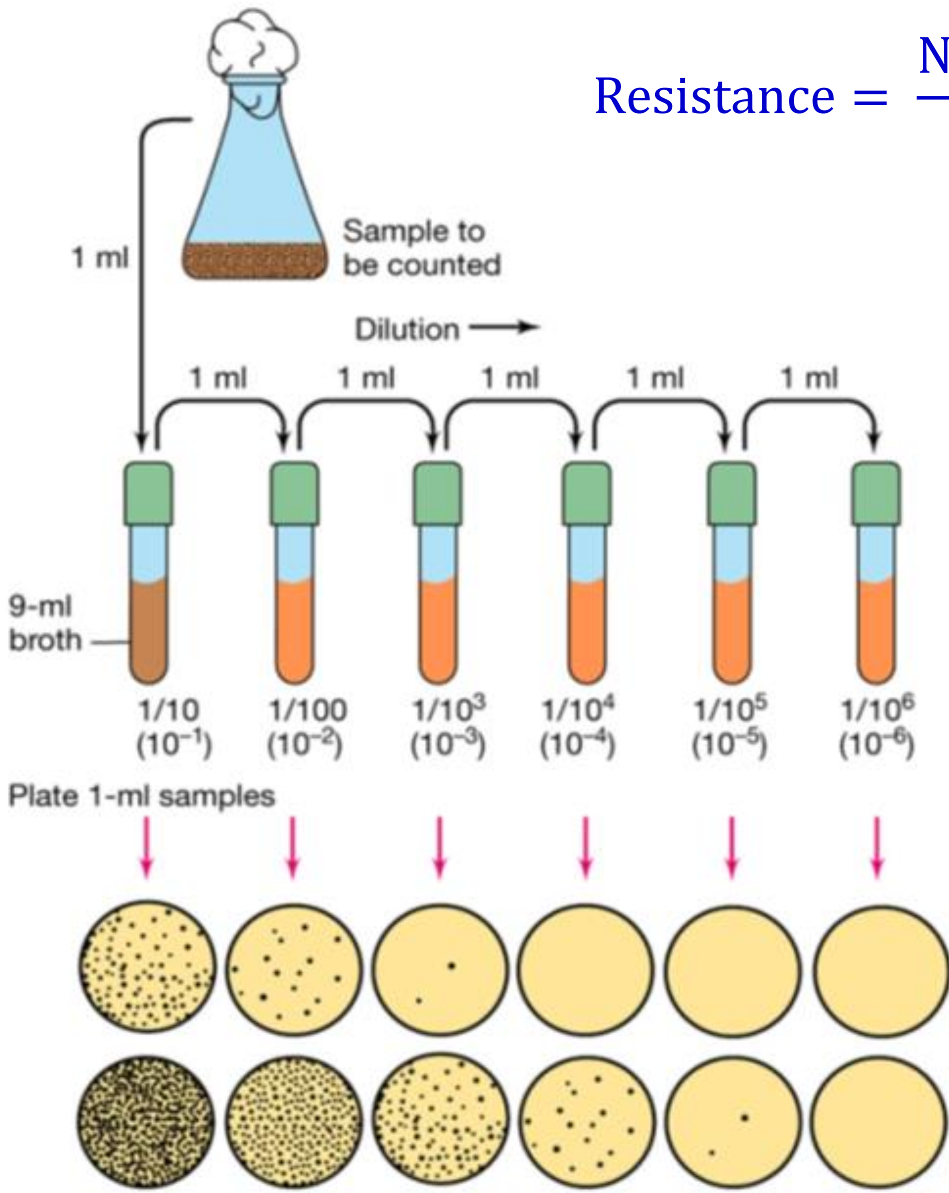




Brock Biology of Microorganisms

Community Triclosan Resistance

$$\text{Resistance} = \frac{\text{Number of CFUs on Triclosan Plates}}{\text{Number of CFUs on Soy Plates}}$$



Triclosan Amended Plates (16mg/L)

Soy Agar Plates

Brock Biology of Microorganisms

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Bacterial Community Composition

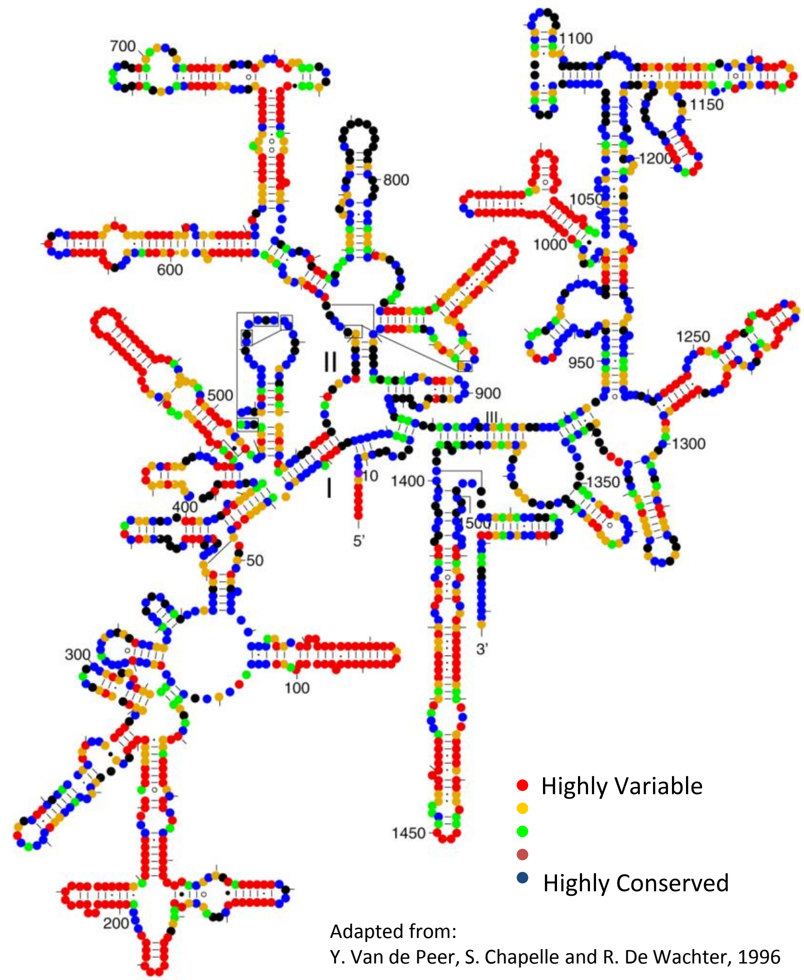
Pyrosequencing

- Developed in 1996
- Enables incredibly high-throughput DNA sequencing
- Generates 700Mb of DNA sequence in a 23 hour run
- Individual reads are between 300-1000 bp
- Many applications

Roche 454 GS-FLX Sequencer

Tag 16S Pyrosequencing

- PCR amplification of V4 region of 16S rRNA gene
- Barcodes (8b) unique to each sample are attached to 5’ end of forward PCR primer
- Amplicons from all samples pooled and sequenced
- Sequences from individual samples can be separated by barcode
- We obtained an average of 10,000 reads per sample
- Sequences processed using MOTHUR (Schloss *et al*., 2009)



Adapted from:
Y. Van de Peer, S. Chapelle and R. De Wachter, 1996

Sediment Triclosan Concentrations

	ng g^{-1} *
Urban River Upstream of WWTP	107 (18)
Urban River Downstream of WWTP	33 (11)
Suburban River Upstream of WWTP	9 (4)
Suburban River Downstream of WWTP	4 (1)
Woodland River	1 (0)

* Limit of detection = 1 ng g^{-1}. Each data point represents mean value (n=5) with standard error values in parentheses.

Drury et al., In Prep

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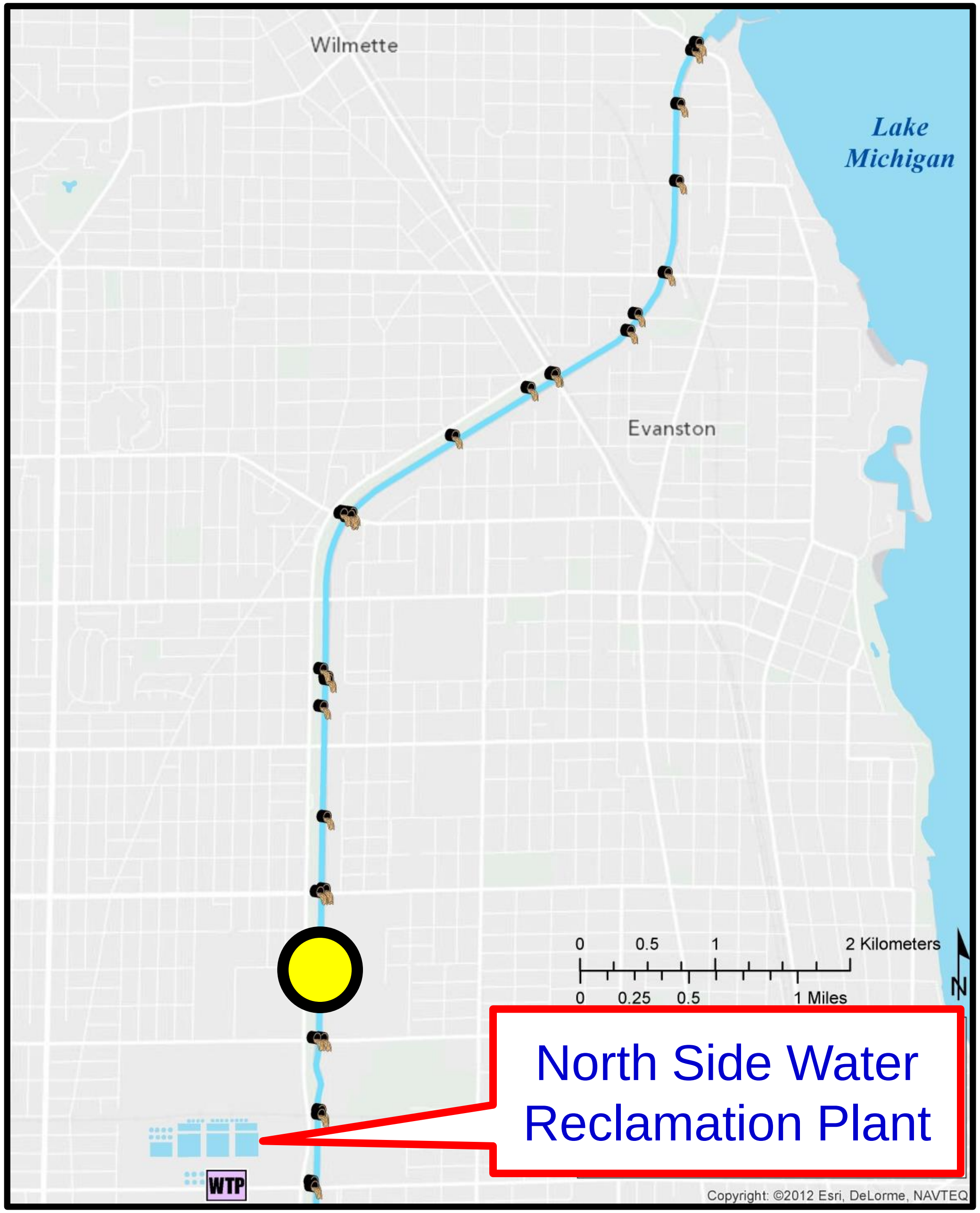
Drury et al., In Prep

Combined Sewer Overflows

- Release untreated wastewater and storm water during high rainfall
- There are 18 CSOs upstream of our Urban Upstream sampling location

Urban Site: North Shore Channel



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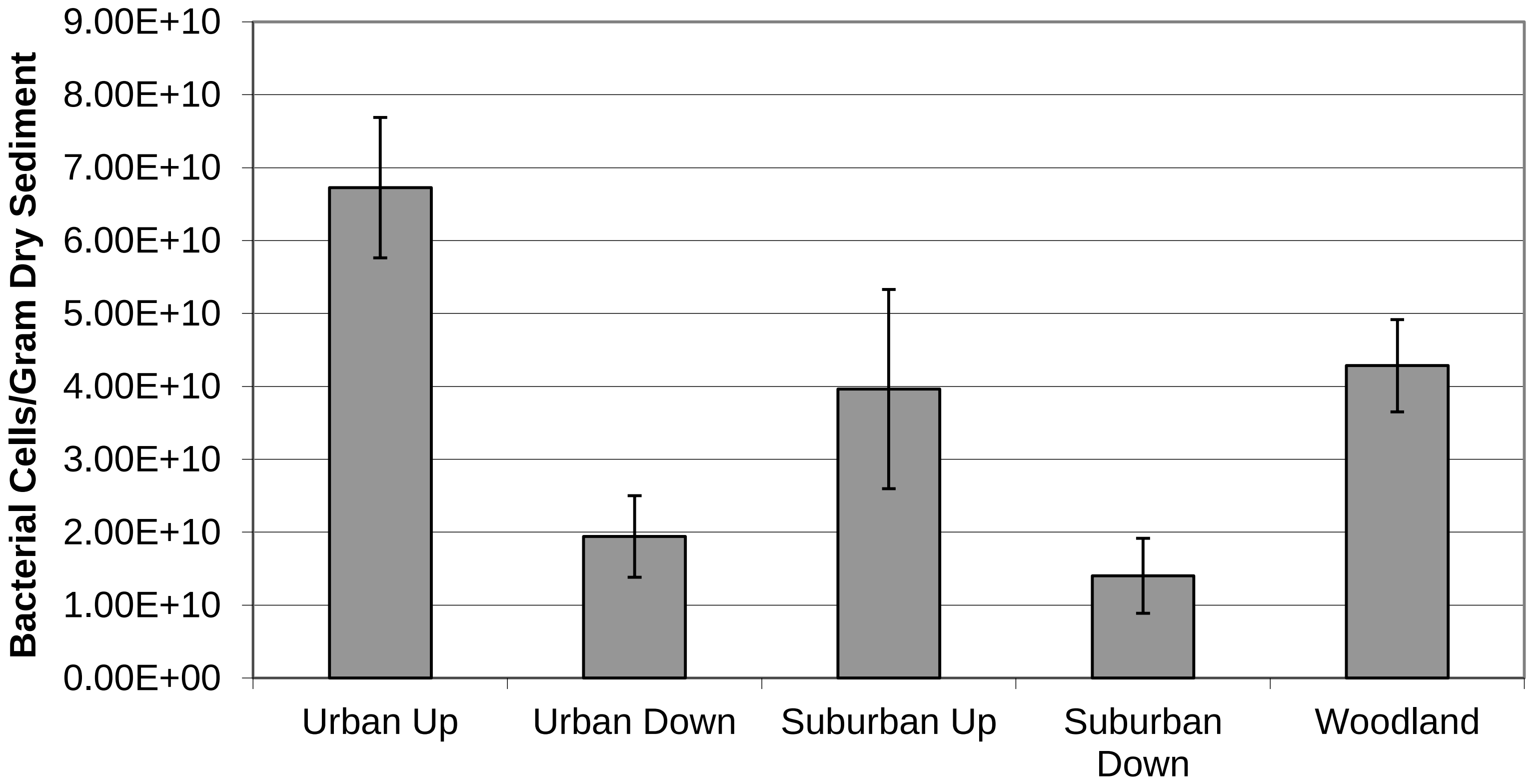
Drury et al., In Prep

Bacterial Abundance Varied by Site



Mean (n=5) +/- Standard Error

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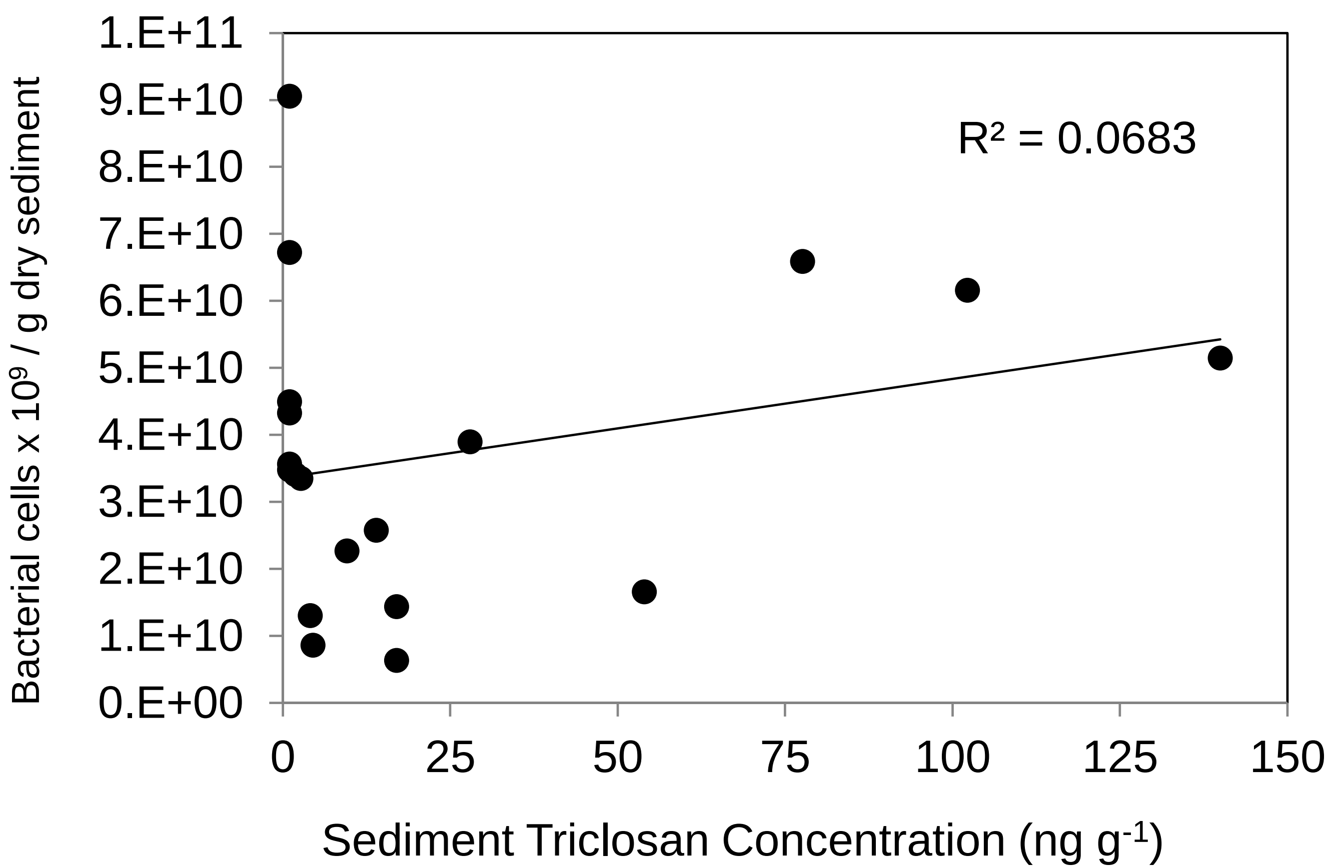
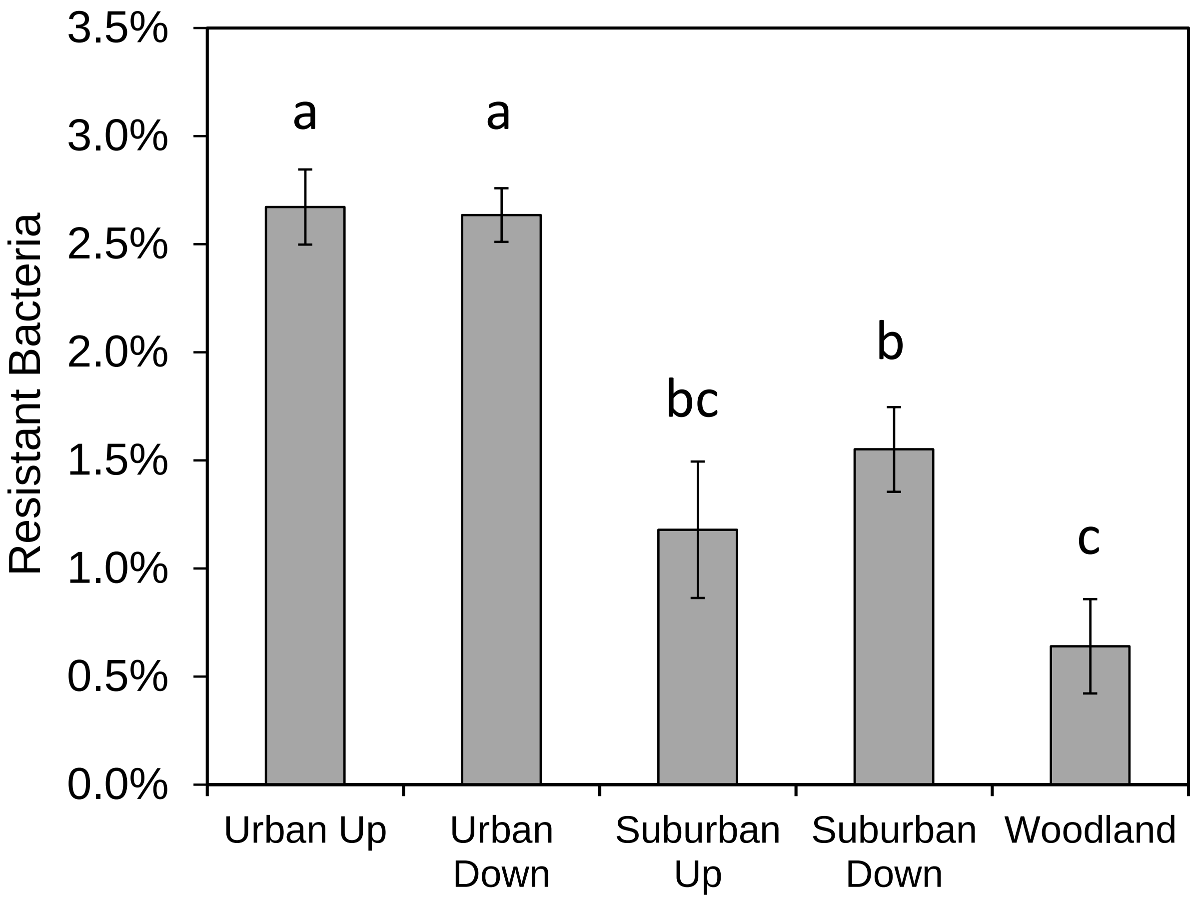
No Relationship Between Triclosan Concentration and Bacterial Abundance



Drury et al., In Prep

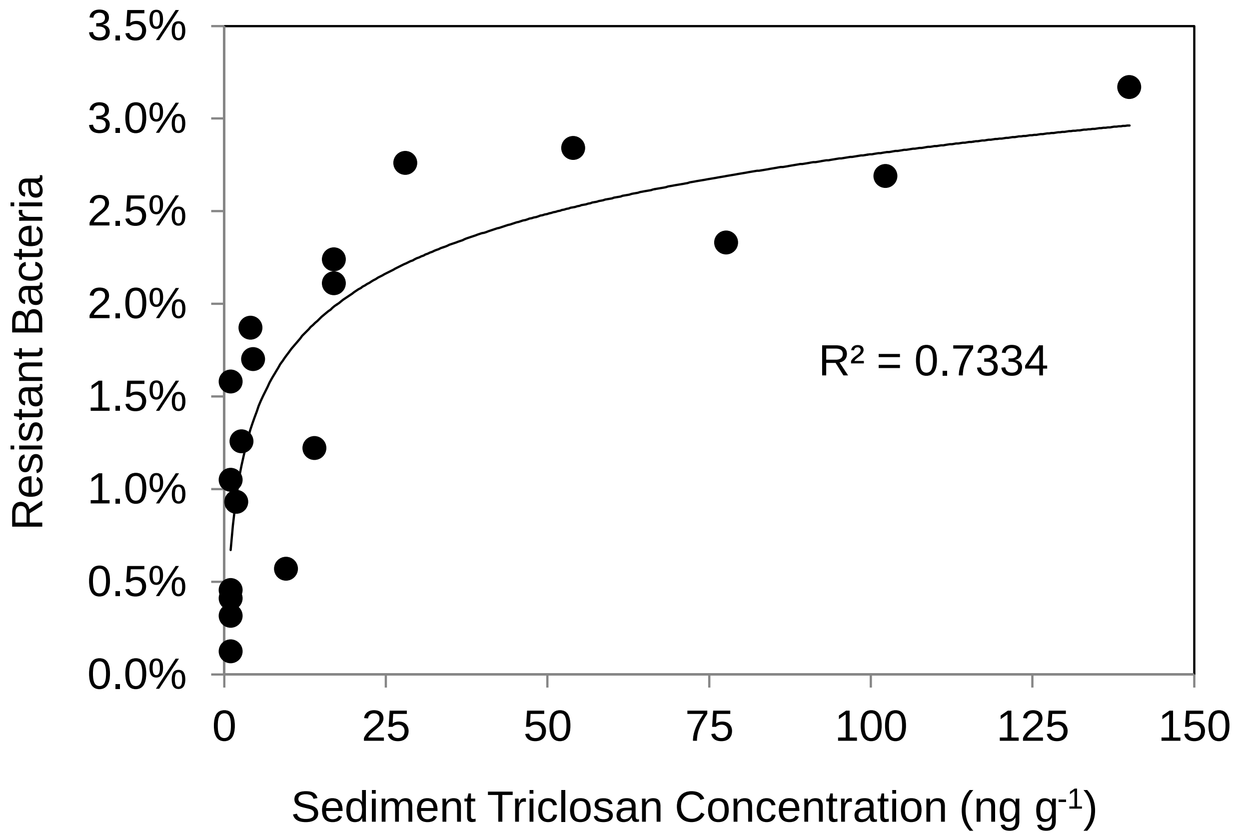
Triclosan Resistance Varied by Site



Mean (n=5) +/- Standard Error

Drury et al., In Prep

Correaltion Between Triclosan Concentration and Resistance of Bacterial Communities



Pearson correlation analysis indicated a significant correlation ($p<0.001$).

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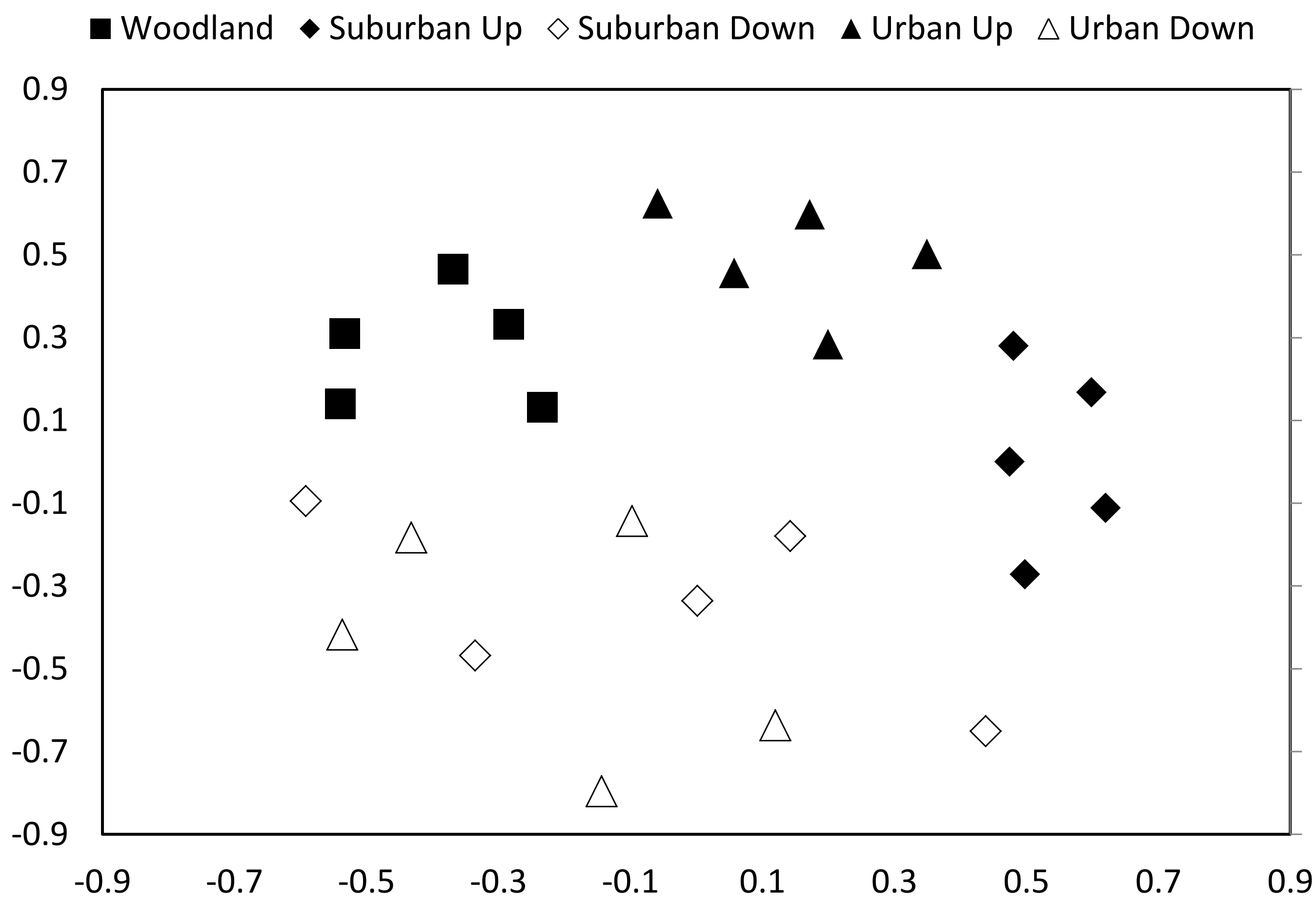
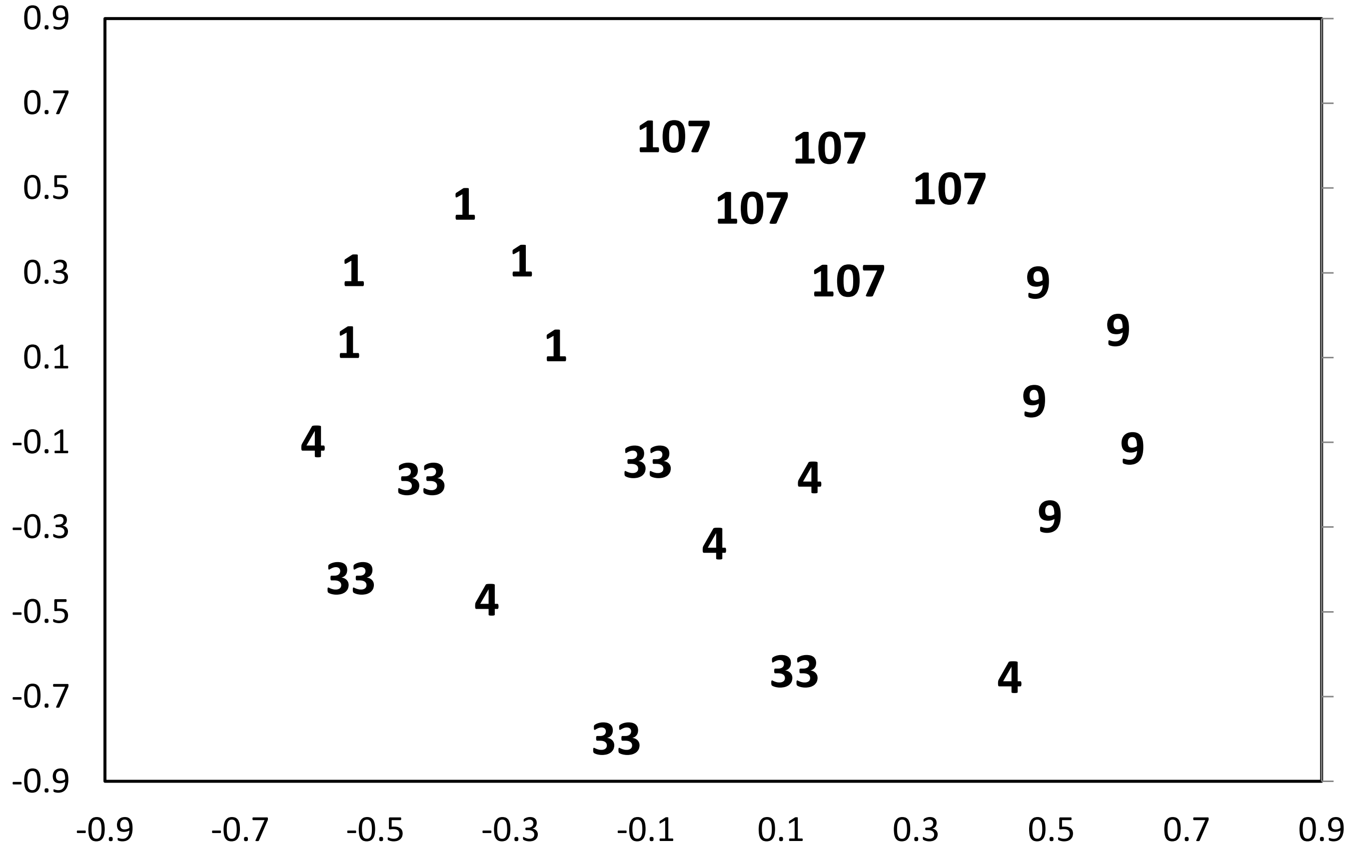
Bacterial Community Composition Varied by Site



MDS Ordination of 16S rRNA Tag Pyrosequencing Data
Total of 214,711 sequences (8,588 seqs per sample)

Bacterial Community Composition Did Not Correlate with Triclosan Concentration



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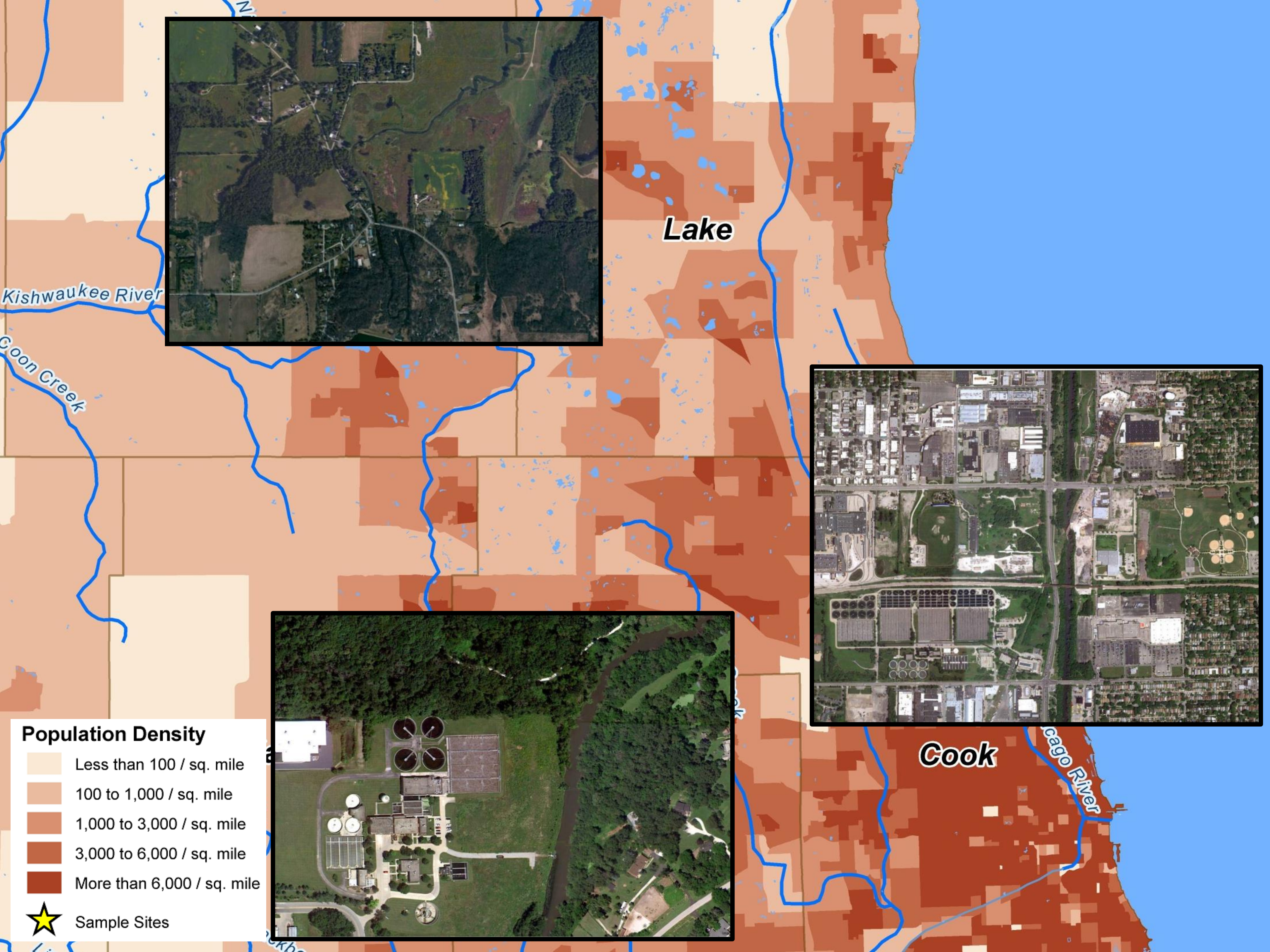

Lake
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Kishwaukee River
Coon Creek
cago River
Population Density
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More than 6,000 / sq. mile
Sample Sites

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Model Stream Experiment

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Mississippi
Montana
KANSAS
Lousiana
Maine
Maryland
Florida
Georgia
Idaho
Illinois
Indiana

Model Stream Experiment

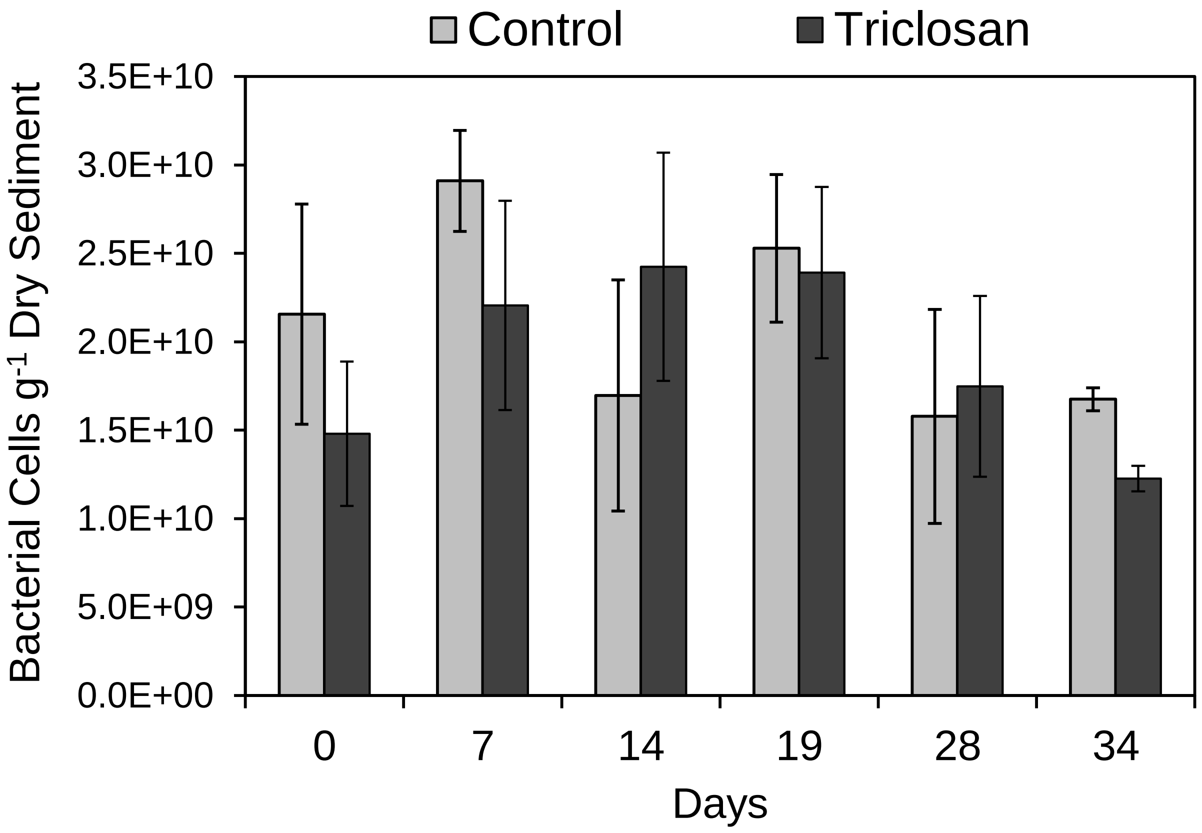
- Model Streams (6)
  - 4 m x 15.5 cm x 15 cm
  - Current maintained by motorized paddle wheels
  - Shaded to block 50% of incoming solar radiation to limit algal growth
- Sediment
  - 0.5kg pea gravel, 9.5kg sand and 66.7g each of shredded red maple, ginkgo and oak leaves (2% organic carbon)
  - Leaves were pre-leached to remove tannins
- Water
  - 60L of dechlorinated tap water
  - Refilled weekly to replace evaporation
- Microbial inoculum
  - 100 g sediment from woodland site

Model Stream Experiment

- Pretreatment
  - Streams were run for two months to allow for adequate colonization of the sediments by microbes.
- Treatment
  - 3 streams received triclosan
    - 722mg of triclosan
      - Amount needed to exceed solubility constant of triclosan (10mg L^{-1}) and bring sediment concentration to 200ng g^{-1}
  - 3 control streams
    - No triclosan
- Sampling
  - Samples collected prior to dosing and every 7 days following treatment

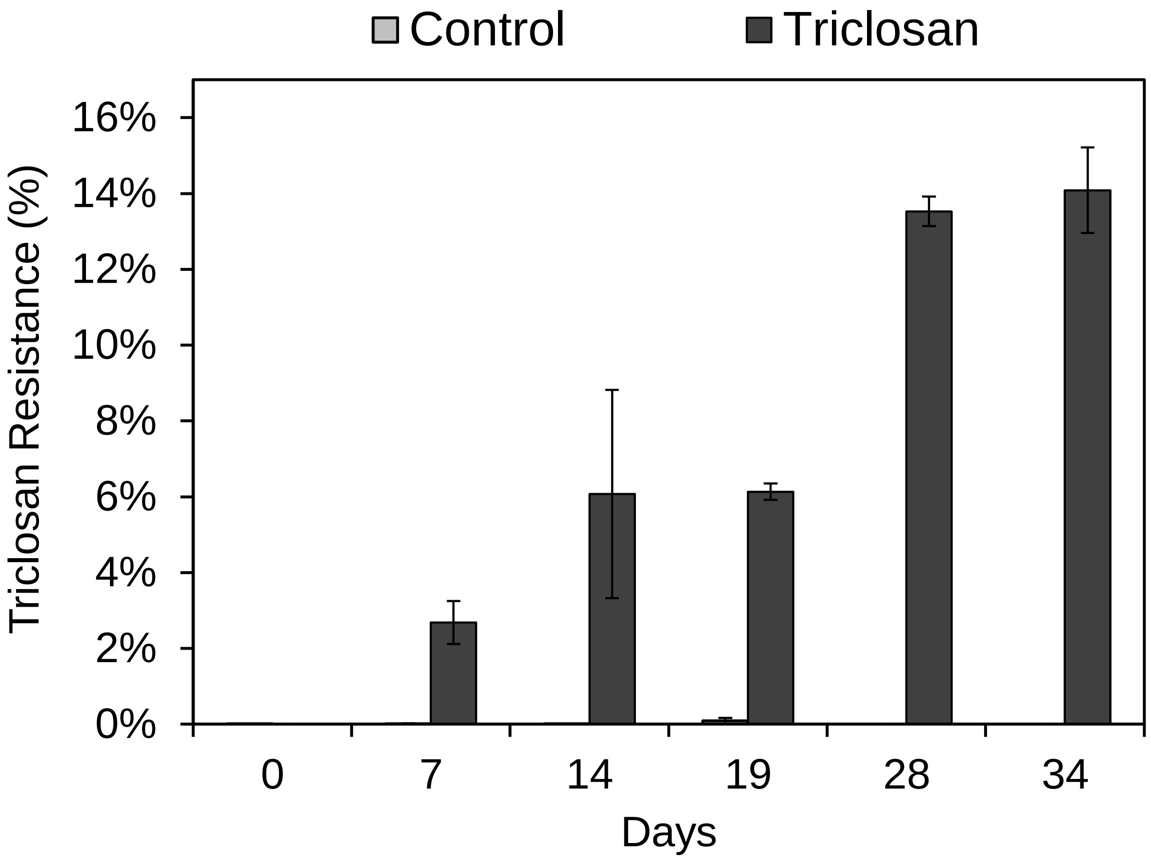
Model Stream Experiment

Triclosan Did Not Affect Bacterial Abundance



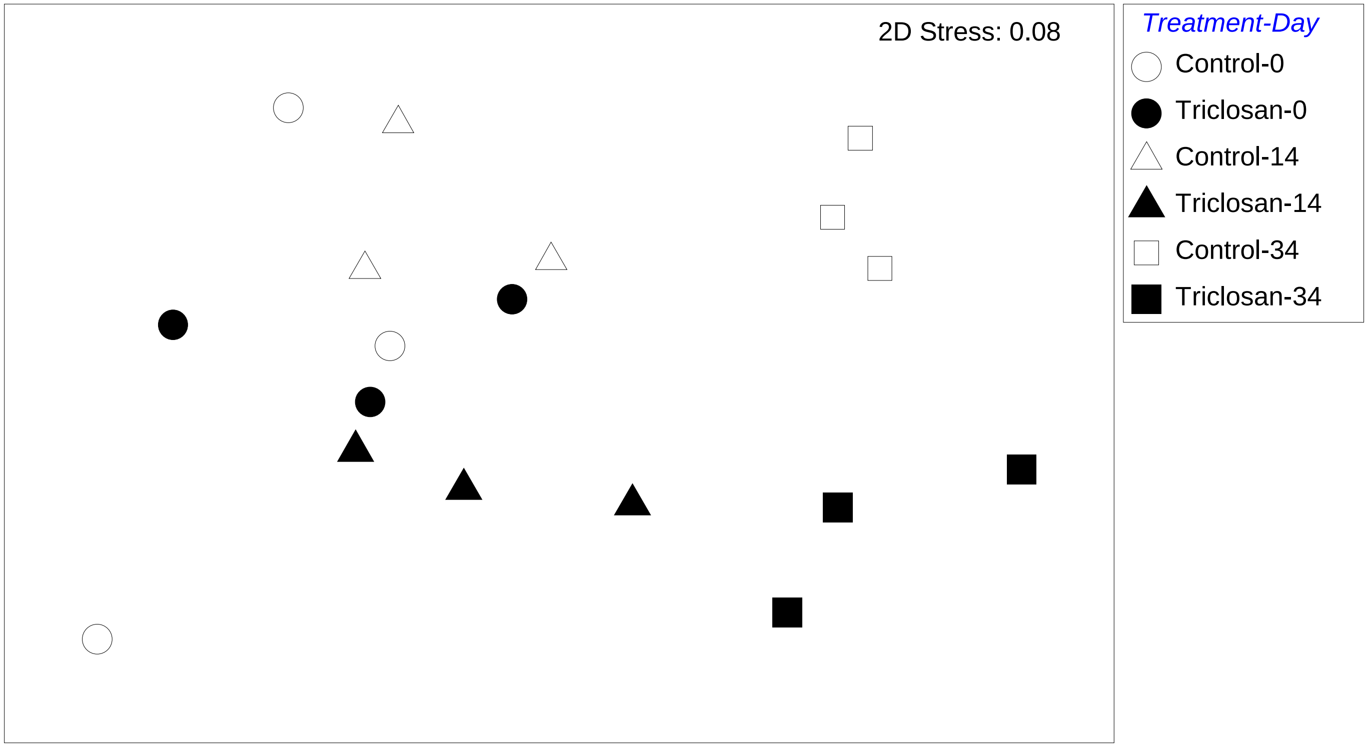
Model Stream Experiment

Triclosan Exposure Increased Bacterial Community Resistance



Model Stream Experiment

Triclosan Exposure Altered
Bacterial Community Composition



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Total of 197,208 sequences (10,956 seqs per sample)

Model Stream Experiment

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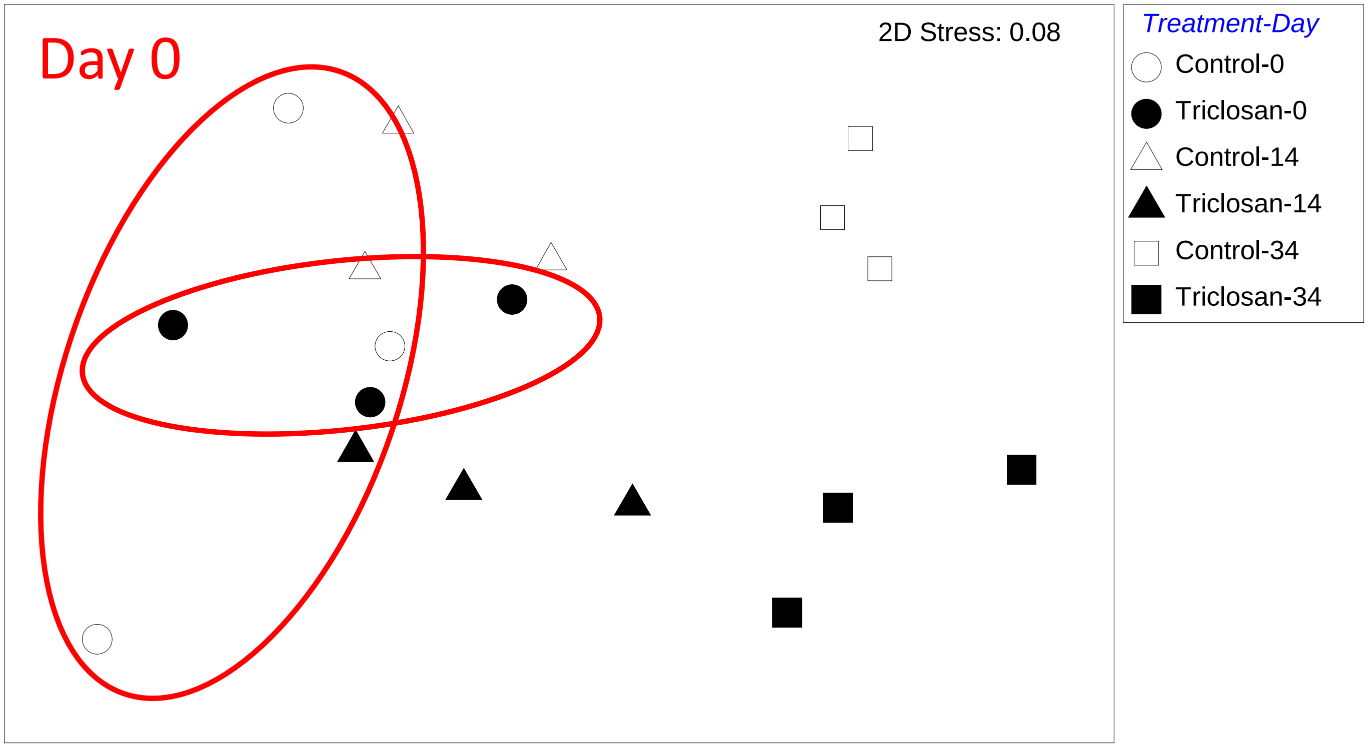

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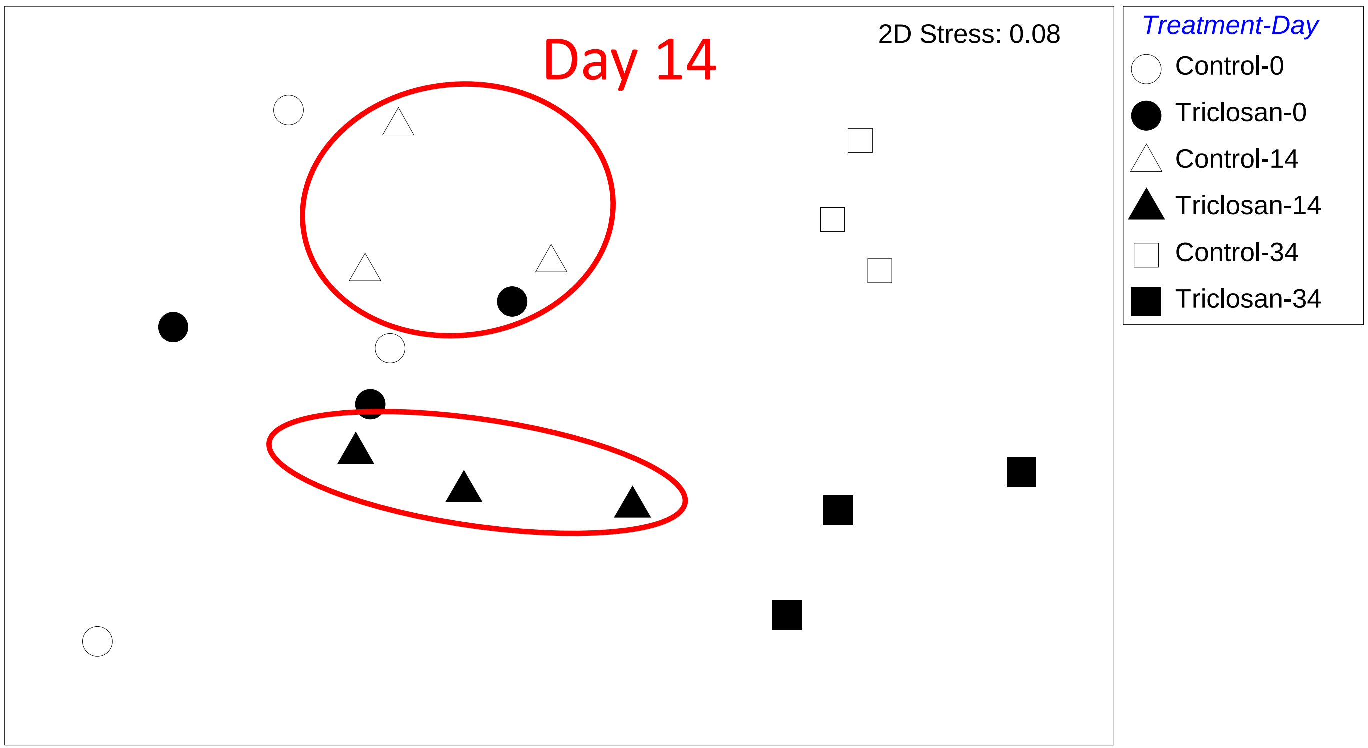

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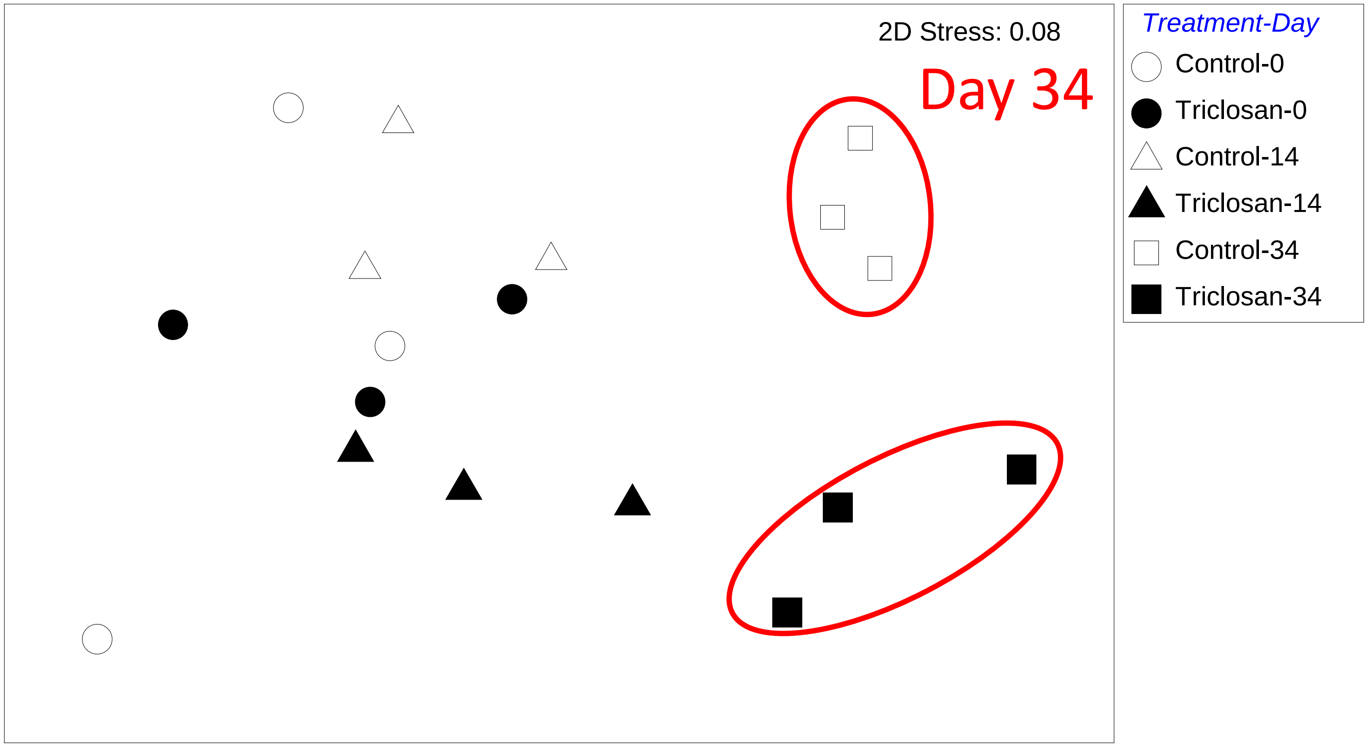

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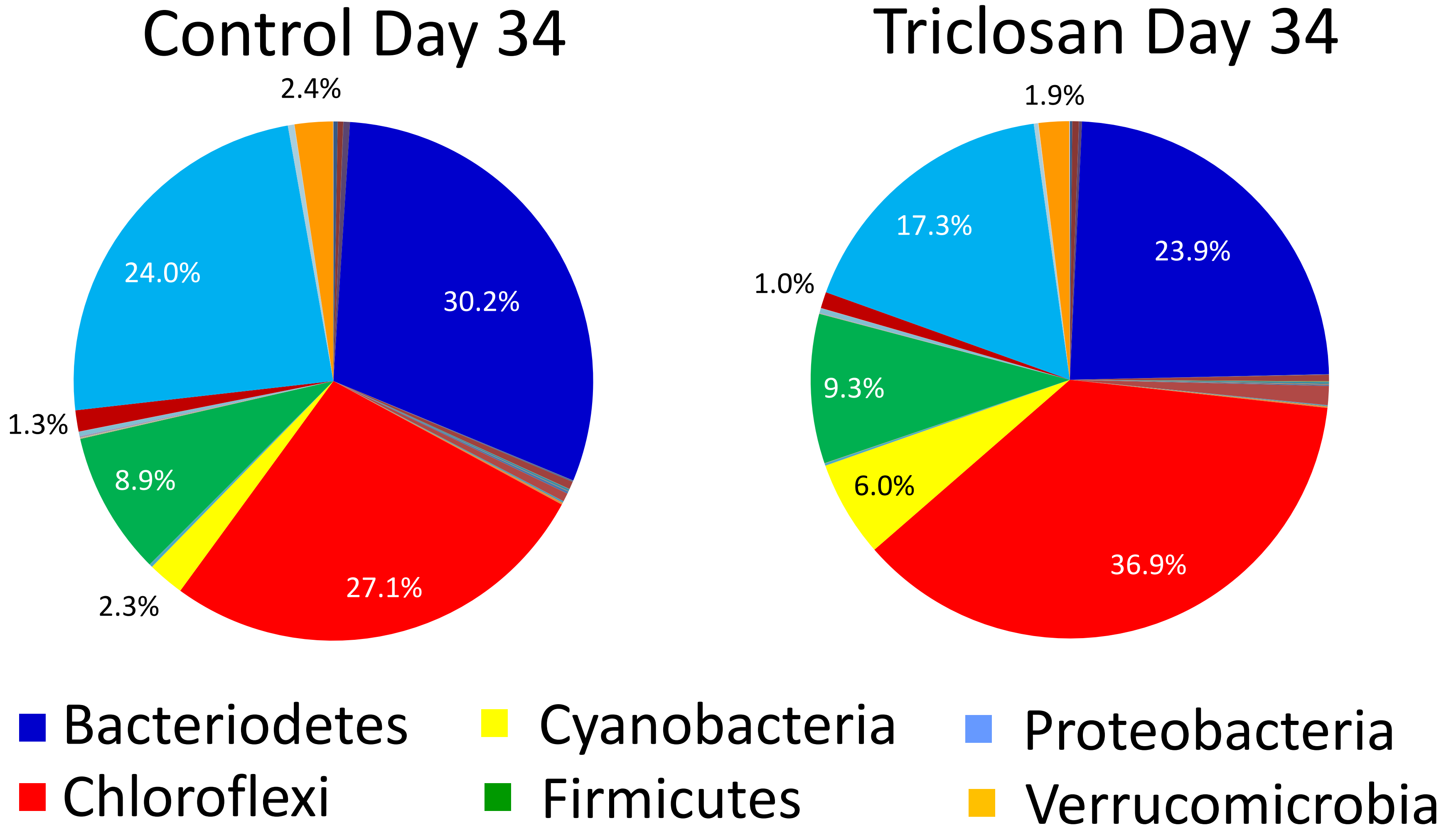
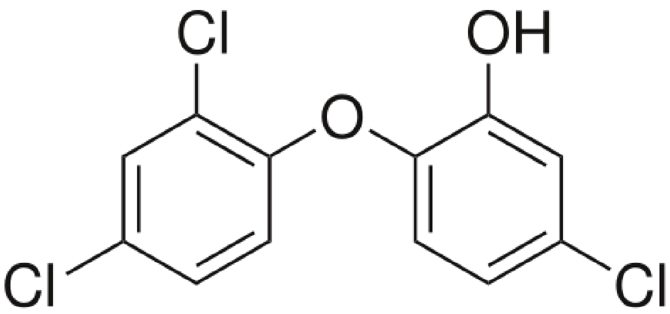


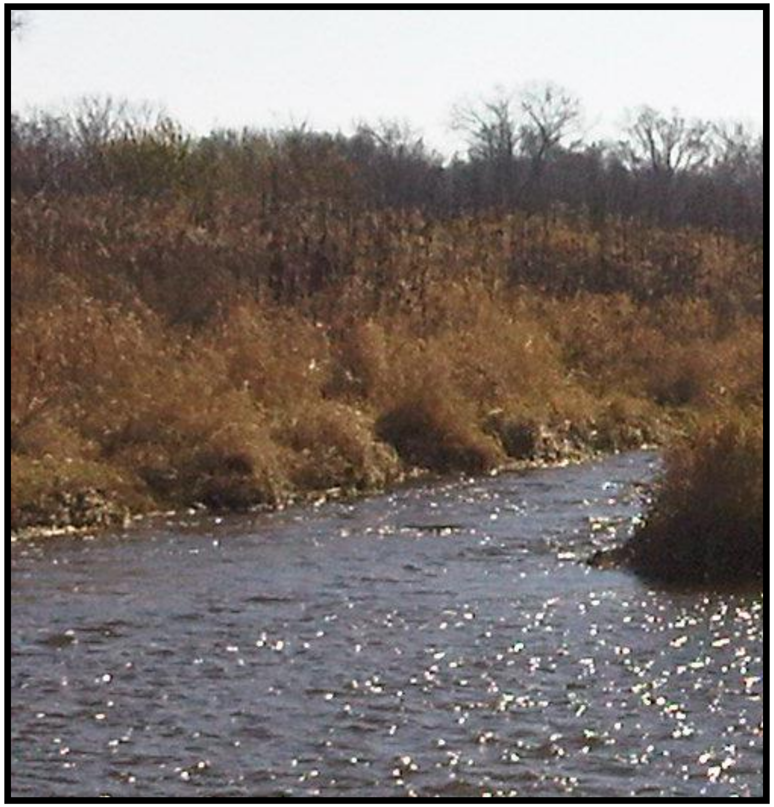
Model Stream Experiment

- Conclusions
  - Sediment bacterial communities adapt quickly to triclosan exposure
  - Triclosan exposure results in
    - Dramatic increases in community triclosan resistance
    - Signficant shifts in bacterial community composition
    - Increase in photosynthetic bacteria

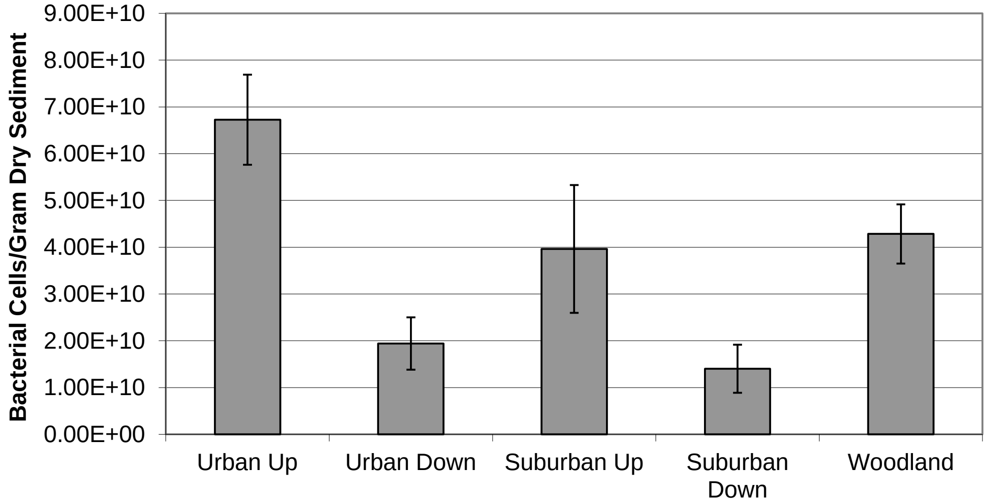
Triclosan in Stream Sediments

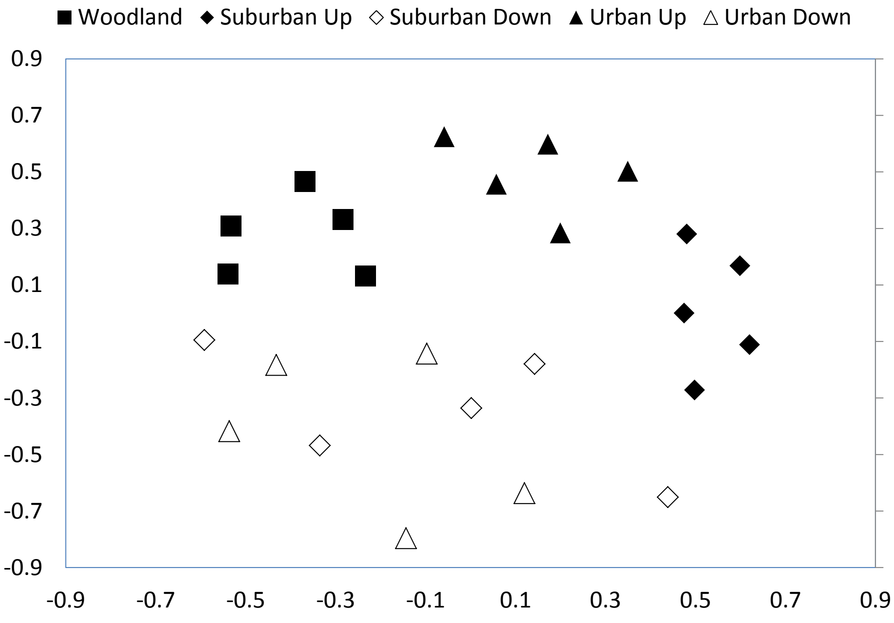
- Future Work
  - Focus on short term responses to triclosan
  - Explore differing sensitivities of microbial taxa to triclosan
  - Focus on functional responses
  - Explore possibility of field amendments of triclosan



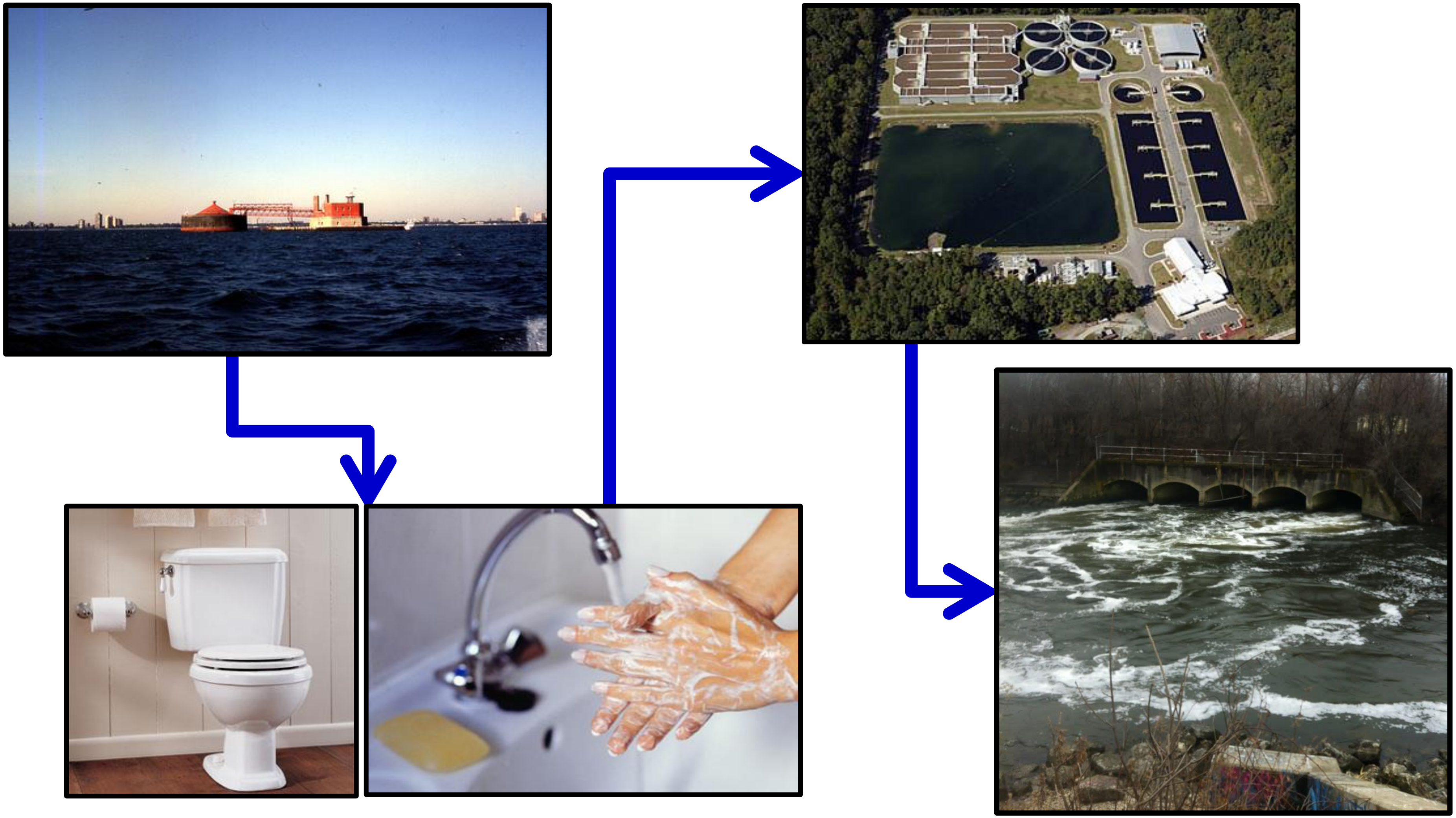


Effects of WWTP Effluent on Sediment Microbial Communities





Most wastewater in the U.S. is treated at centralized wastewater treatment plants (WWTPs)



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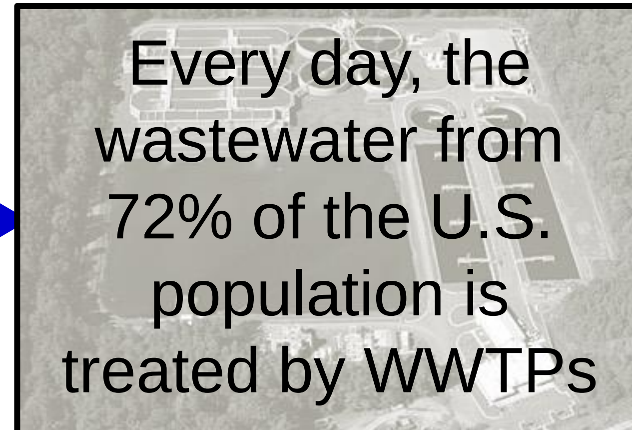
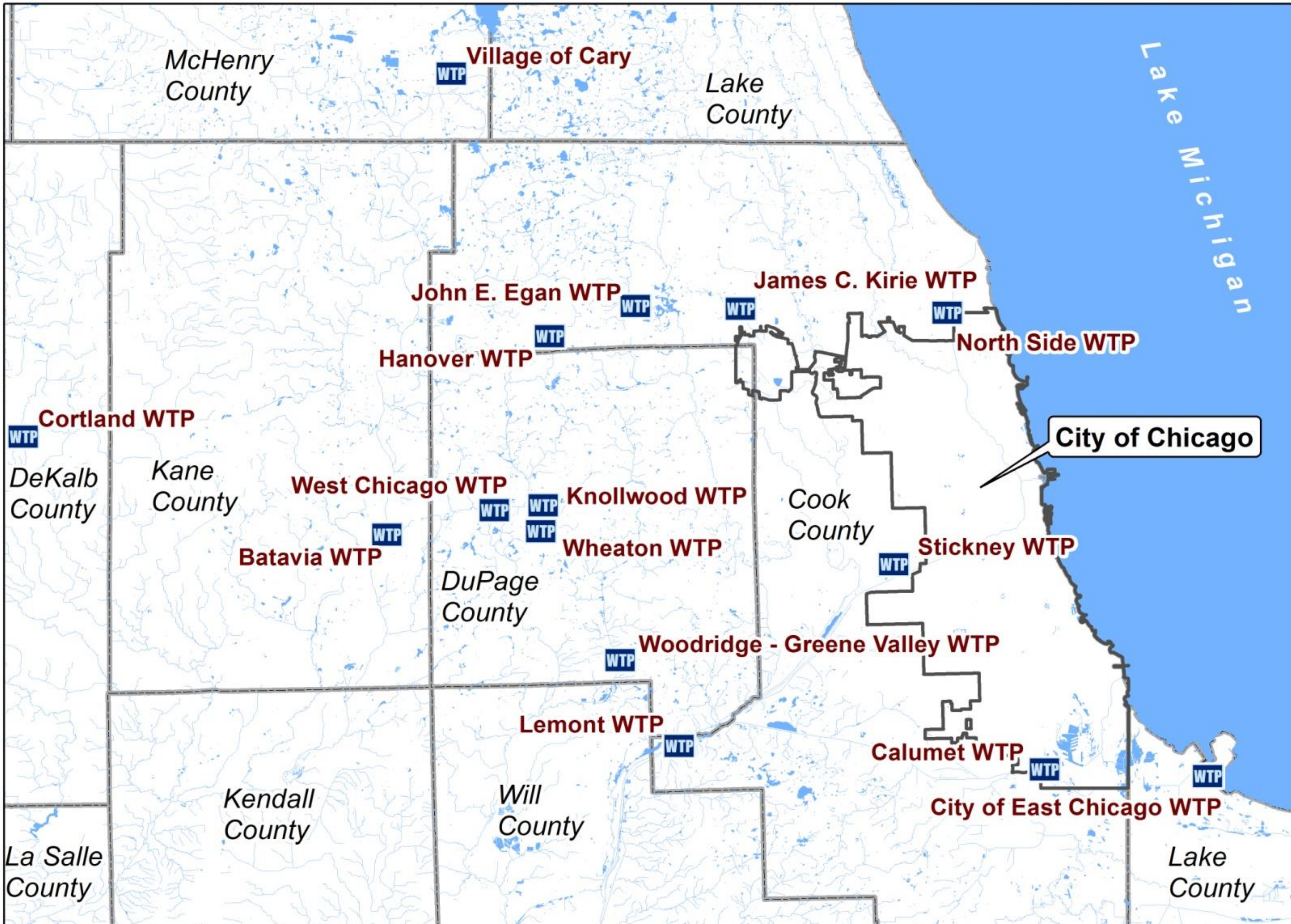


Most wastewater in the U.S. is treated at centralized wastewater treatment plants (WWTPs)

Every day, the wastewater from 72% of the U.S. population is treated by WWTPs

Every day, 42 billion gallons of treated wastewater are discharged to U.S. rivers and streams

There are 15 WWTPs in the Chicago Metropolitan Region



Map by David J. Treering, Center for Urban Environmental Research & Policy, Loyola University Chicago, 2012

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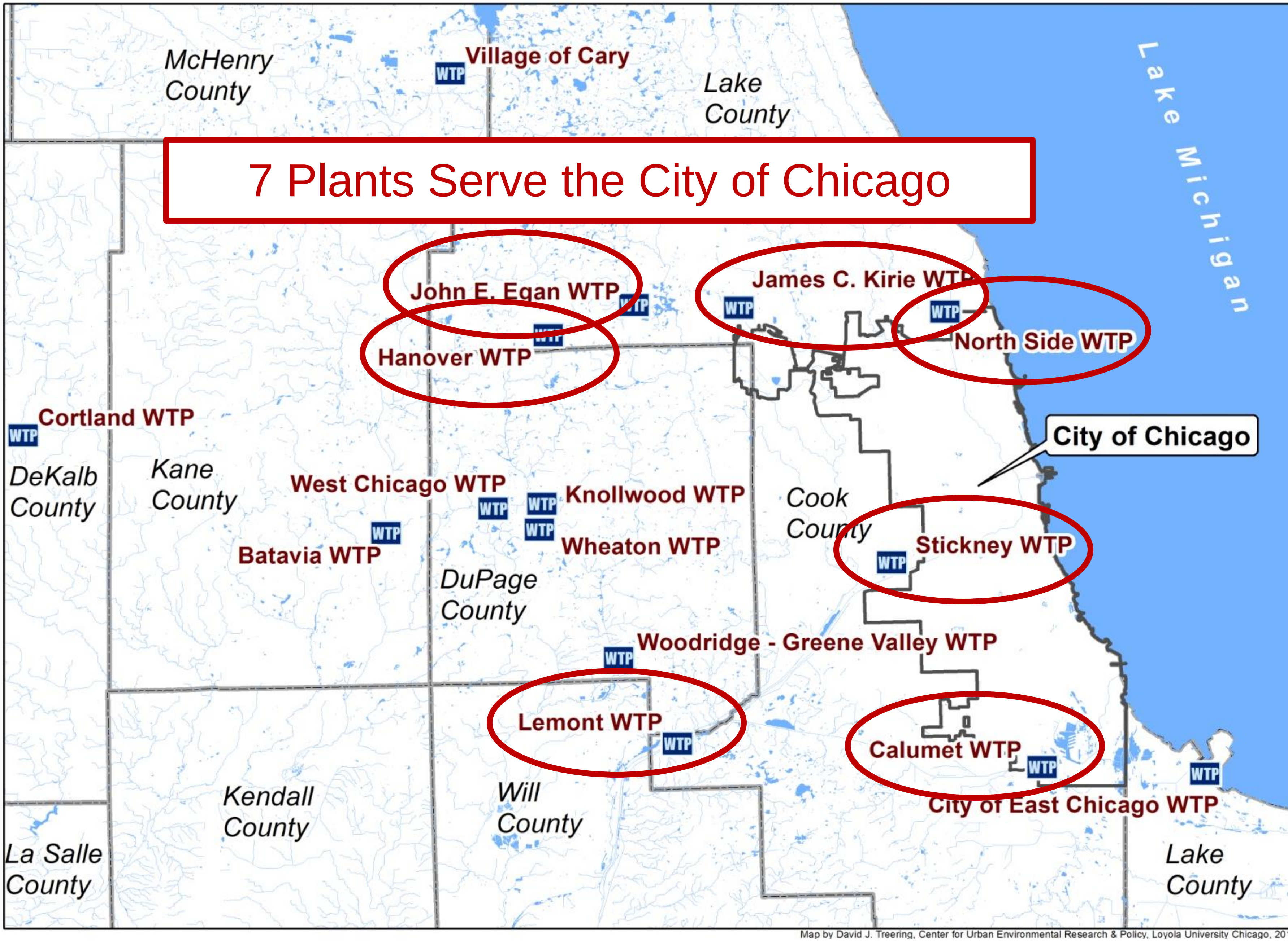
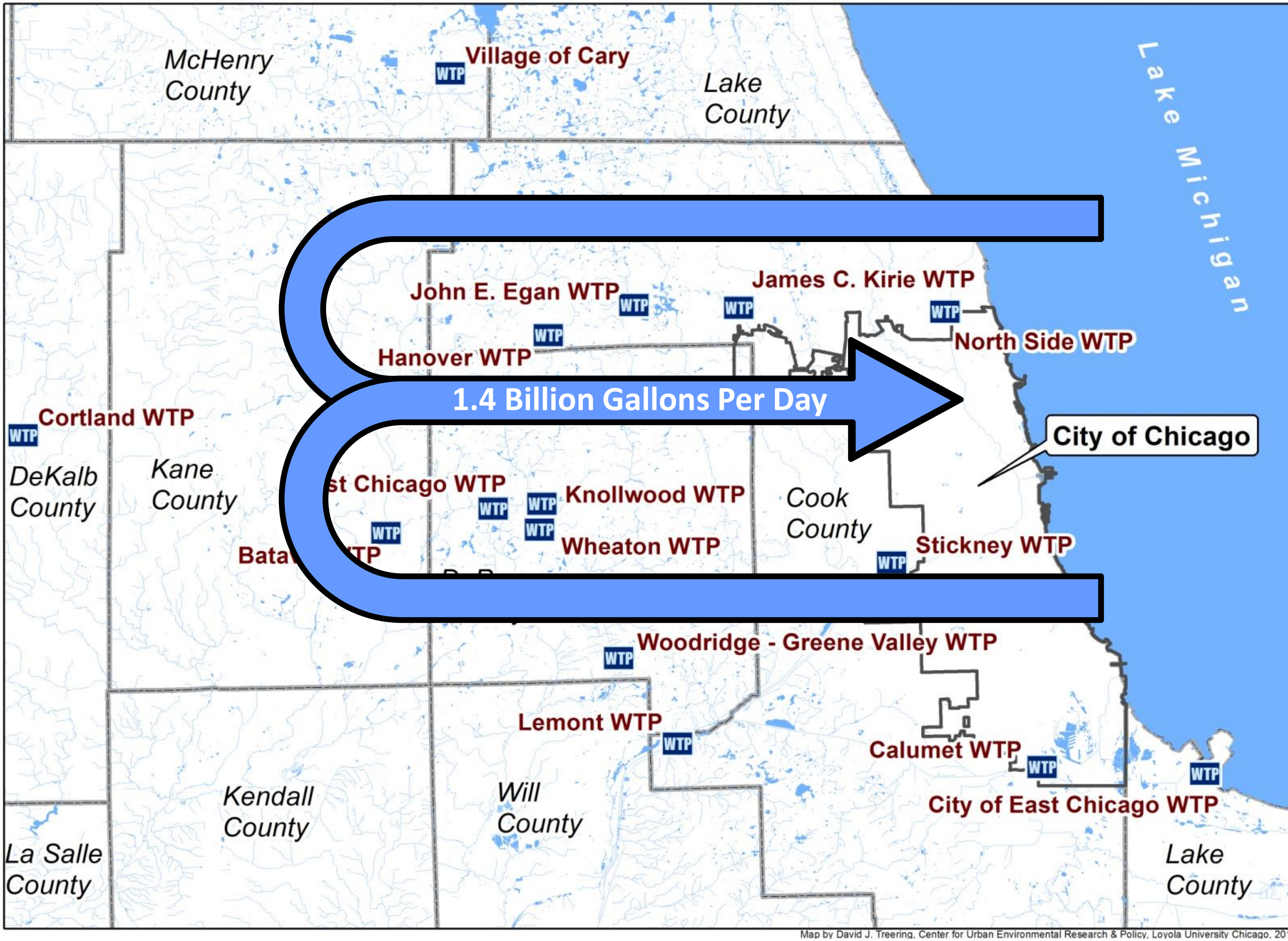


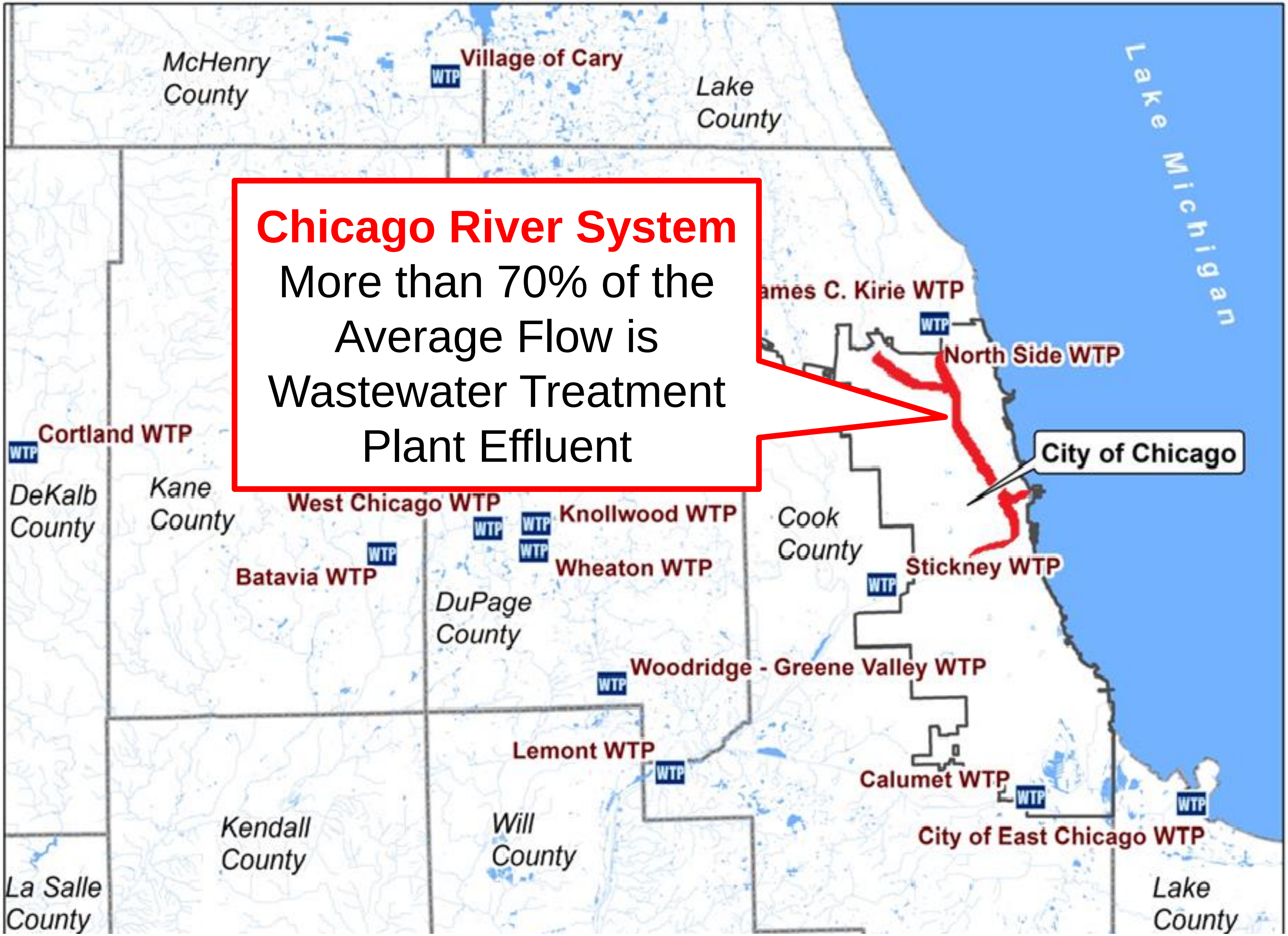
Chicago WWTPs process a high volume of water

Plants Serving Chicago	Average Flow (MGD)*
Stickney WRP	787
Calumet WRP	287
North Side WRP	246
Kirie WRP	46
Egan WRP	30
Hanover Park WRP	9
Lemont WRP	3
All Plants	**1.4 Billion Gallons per Day**

* 2008 Operating Data



McHenry County
Village of Cary
Lake County
Lake Michigan
John E. Egan WTP
James C. Kirie WTP
North Side WTP
Hanover WTP
1.4 Billion Gallons Per Day
Cortland WTP
City of Chicago
DeKalb County
Kane County
st Chicago WTP
Knollwood WTP
Cook County
Wheaton WTP
Stickney WTP
Bata
TP
Woodridge - Greene Valley WTP
Lemont WTP
Calumet WTP
Kendall County
Will County
City of East Chicago WTP
La Salle County
Lake County
Map by David J. Treering, Center for Urban Environmental Research & Policy, Loyola University Chicago, 2012



Chicago River System
More than 70% of the Average Flow is Wastewater Treatment Plant Effluent
McHenry County
Village of Cary
Lake County
Lake Michigan
ames C. Kirie WTP
North Side WTP
City of Chicago
Cortland WTP
DeKalb County
Kane County
West Chicago WTP
Knollwood WTP
Wheaton WTP
Batavia WTP
Cook County
Stickney WTP
DuPage County
Woodridge - Greene Valley WTP
Lemont WTP
Calumet WTP
City of East Chicago WTP
Kendall County
Will County
La Salle County
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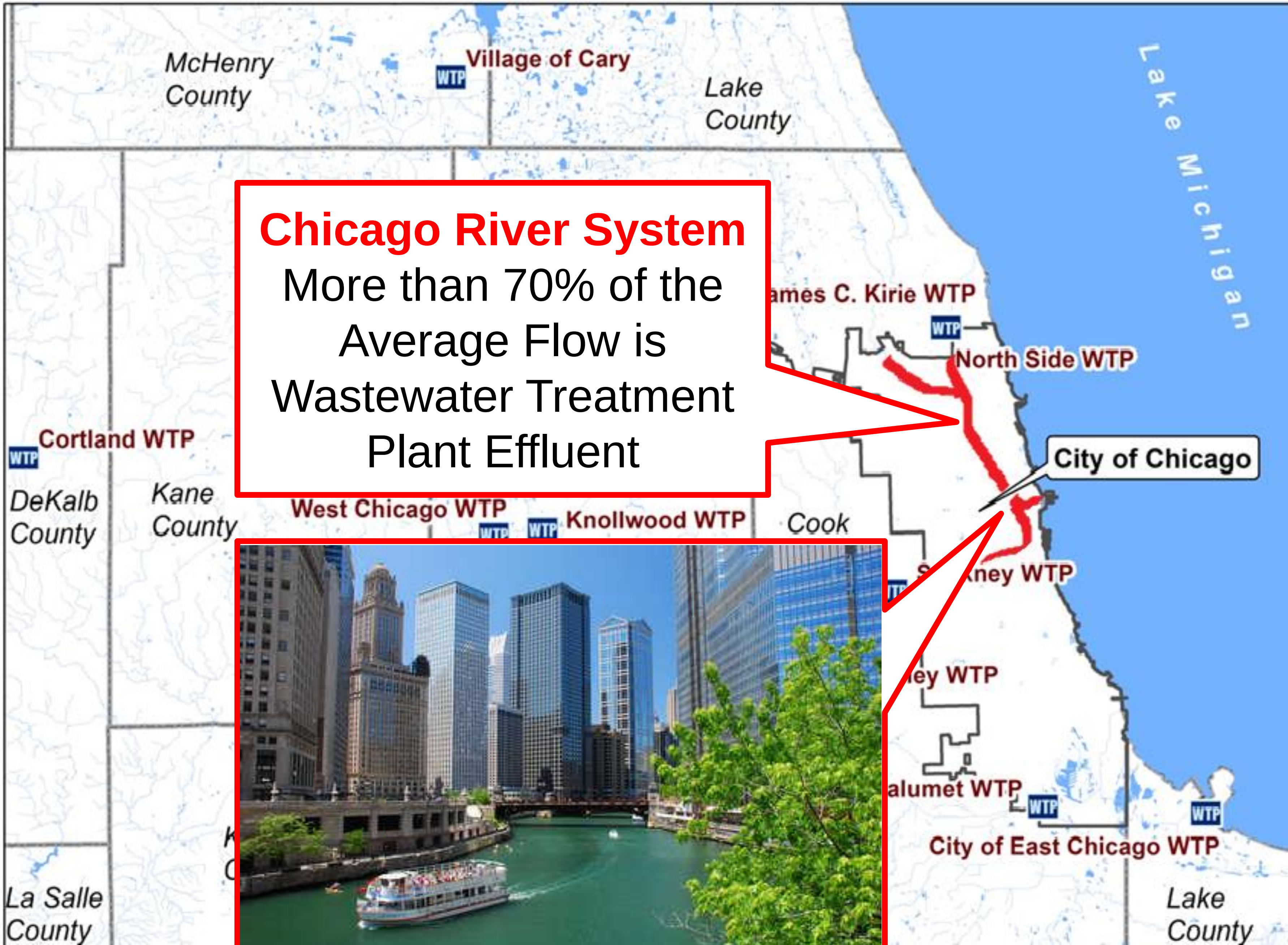

Chicago River System

More than 70% of the Average Flow is Wastewater Treatment Plant Effluent

Some aspects of WWTP effluent impacts on streams have been investigated

- **Pathogen Release**
  - Grimes et al. 1984; Harwood et al. 2005; Castro-Hermida et al. 2008
- **Water Quality / Nutrient Loading**
  - Waiser *et al.* 2011; Jarvie 2006; Smith *et al*. 1999; Haggard *et al*. 2001
- **Eutrophication**
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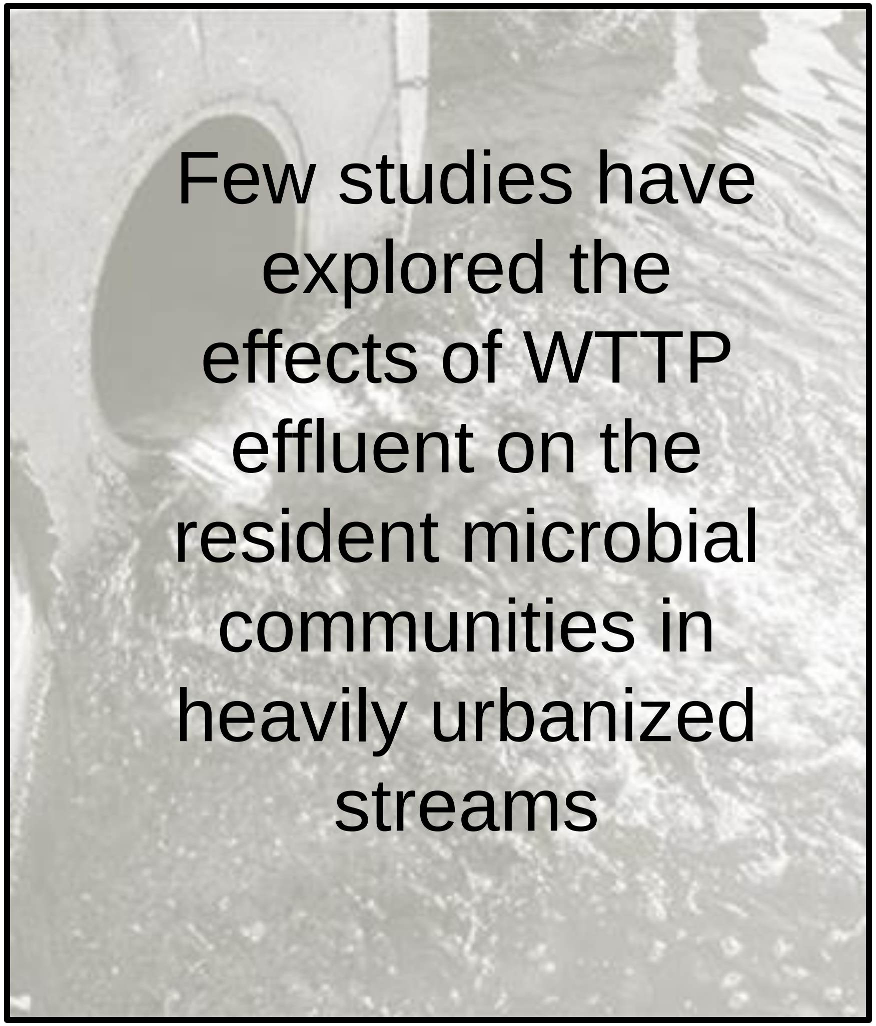
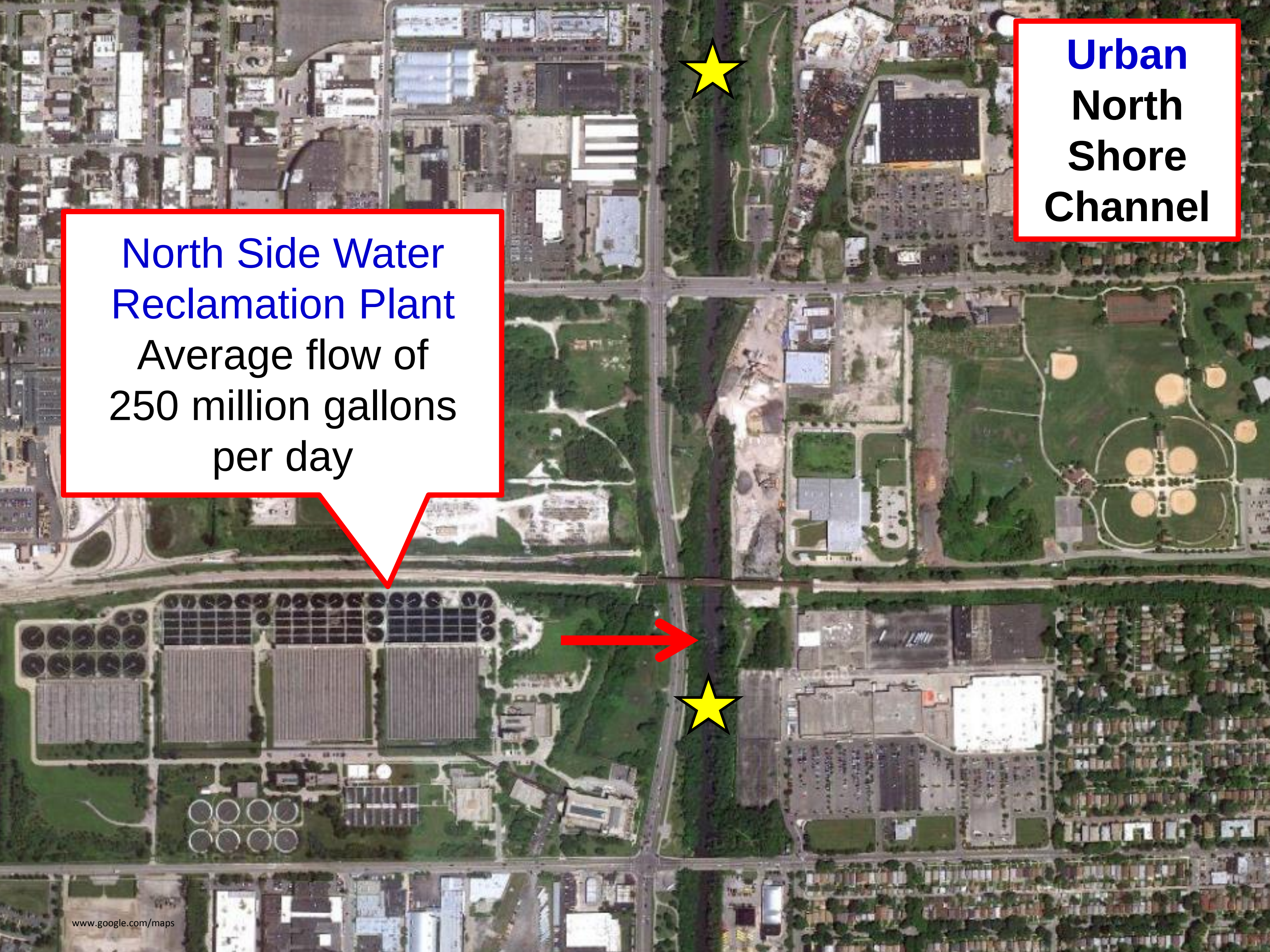
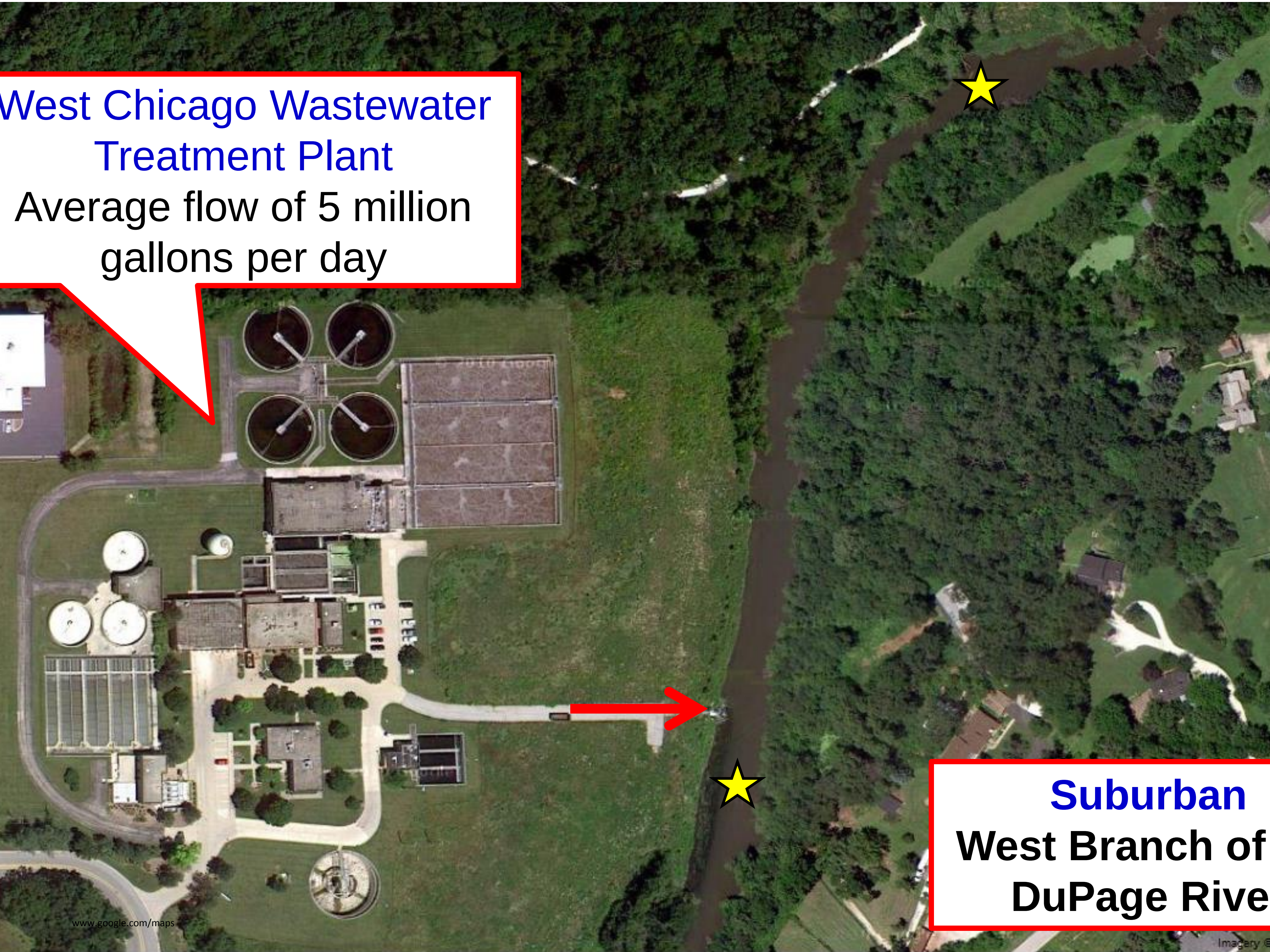



Urban
North
Shore
Channel
North Side Water
Reclamation Plant
Average flow of
250 million gallons
per day
www.google.com/maps



West Chicago Wastewater Treatment Plant
Average flow of 5 million gallons per day
Suburban
West Branch of DuPage Rive
www.google.com/maps

Sampling Site Characteristics

Chemical Characteristics

	Value (SE)			
	Suburban Upstream	**Suburban Downstream**	**Urban Upstream**	**Urban Downstream**
Water Column DOC (mg/L)	6.652 (0.052)	5.782 (0.306)	2.408 (0.085)	3.947 (0.072)
Water Column NH_4 (mg/L)	0.060 (0.003)	<0.02	0.138 (0.007)	0.236 (0.005)
Water Column NO_3^- (mg/L)	2.742 (0.140)	4.662 (0.492)	0.232 (0.002)	4.696 (0.206)
Water Column PO_4^{3-} (mg/L)	0.268 (0.006)	0.466 (0.035)	0.003 (0.000)	0.410 (0.019)
Sediment Organic Material (%)	8.70 (1.20)	1.58 (0.12)	5.89 (0.43)	2.00 (0.21)

Each data point is mean (n=5) with standard error in parentheses

Drury et al., 2013, *Appl Environ Microbiol*

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* Indicates significant effect of habitat ($p<0.05$)

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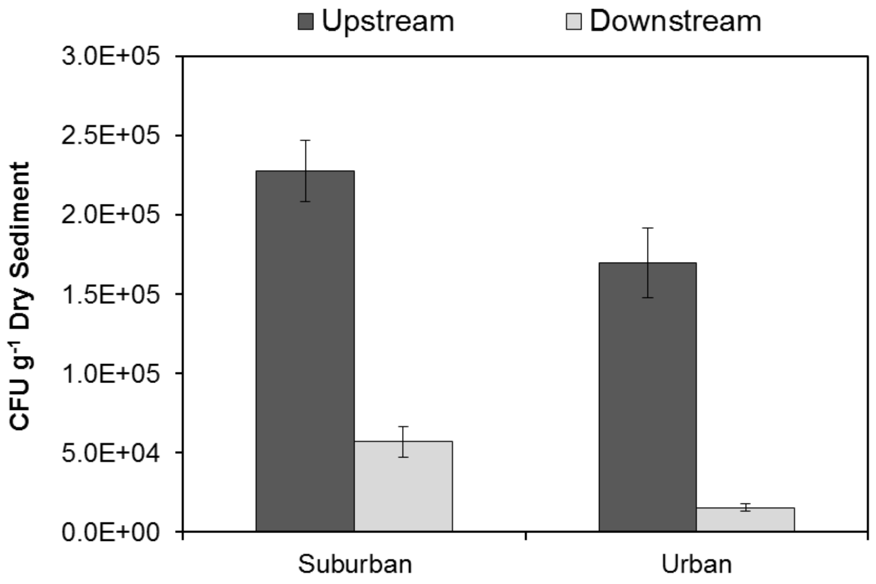
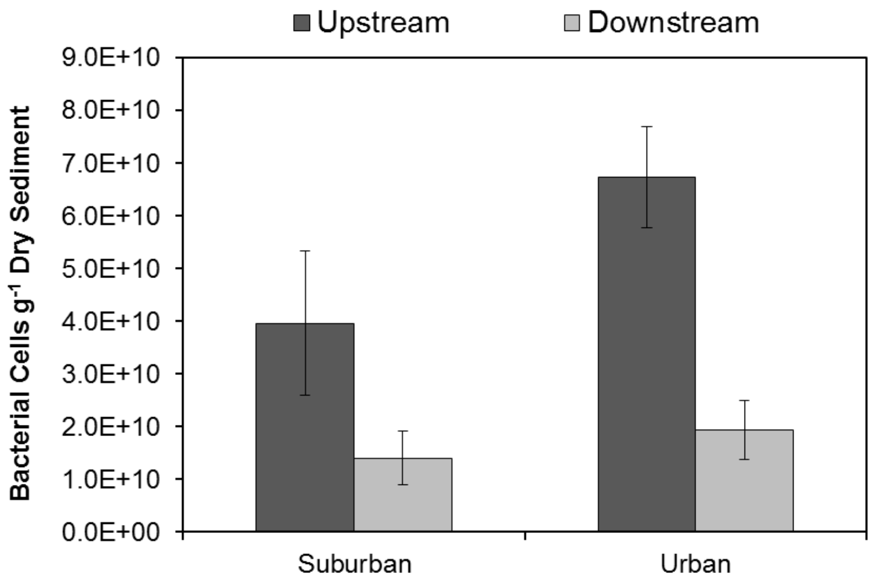
Drury et al., 2013, *Appl Environ Microbiol*



Heterotrophic Plate Counts

- Significant Habitat Effect
- Significant Effluent Effect
- No Interaction

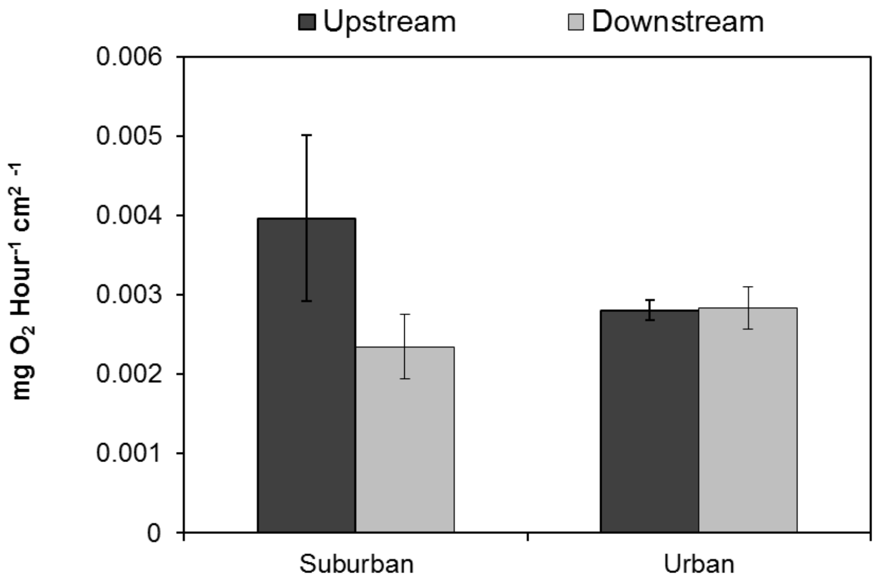


Direct Bacterial Cell Counts

- No Significant Habitat Effect
- Significant Effluent Effect
- No Interaction

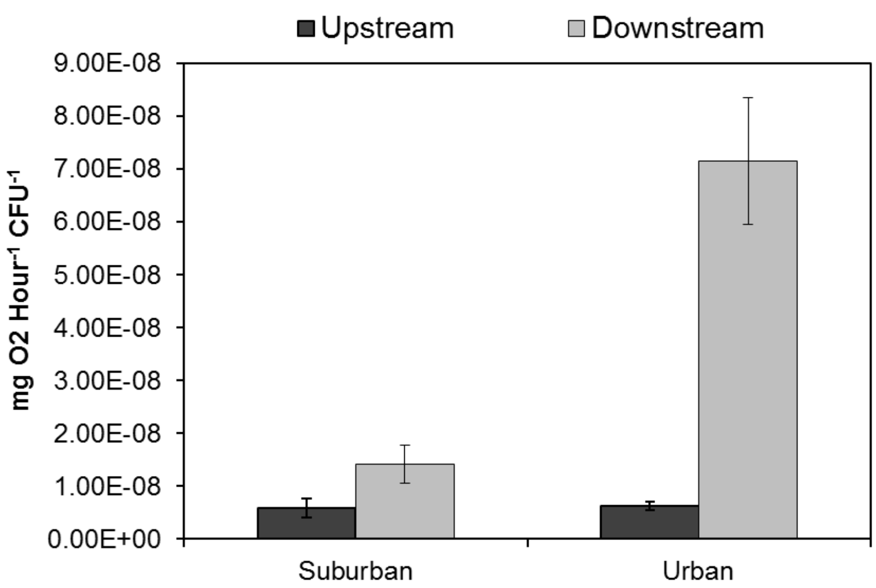
Mean (n=5) +/- Standard Error

Drury et al., 2013, *Appl Environ Microbiol*



Respiration

- No Significant Habitat Effect
- No Significant Effluent Effect
- No Interaction



Per Cell Respiration Rates

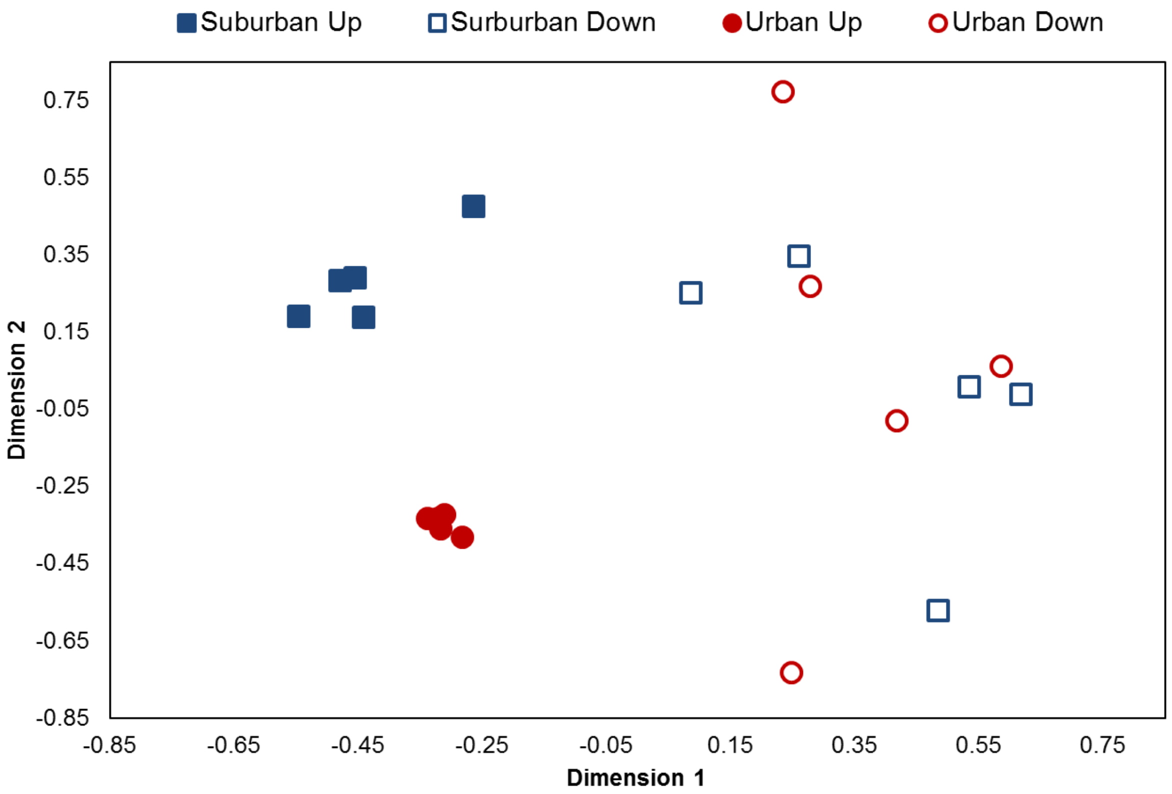
- Significant Habitat Effect
- Significant Effluent Effect
- Significant Interaction

Mean (n=5) +/- Standard Error

Drury et al., 2013, *Appl Environ Microbiol*

Community Structure

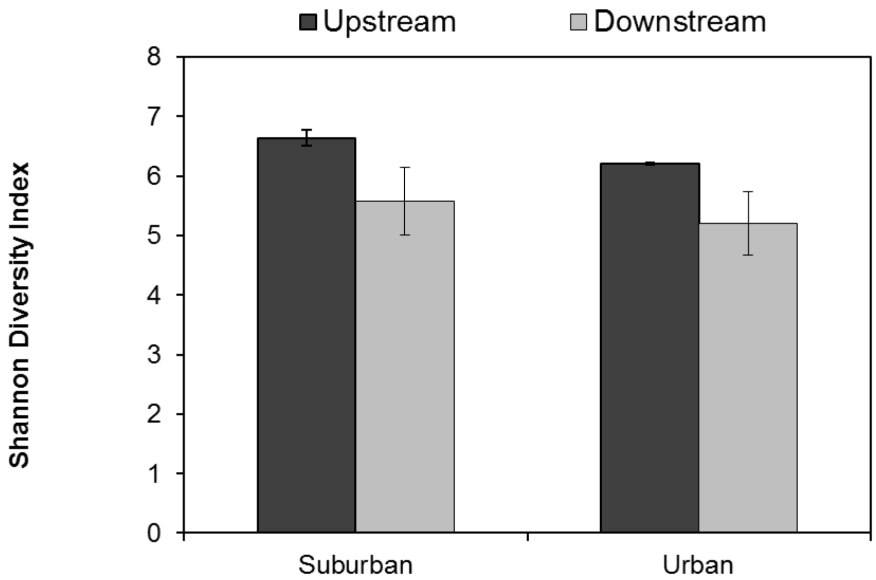
Nonmetric Multidimensional Scaling Ordination of
16S Tag Pyrosequencing Data



AMOVA Analysis

	Value
	P-value
Suburban Up vs Urban Up	<0.001
Suburban Up vs Suburban Down	0.003
Urban Up vs Urban Down	<0.001
Suburban Down vs Urban Down	0.982
Suburban Up vs Urban Down	0.003
Interaction Effect	<0.001

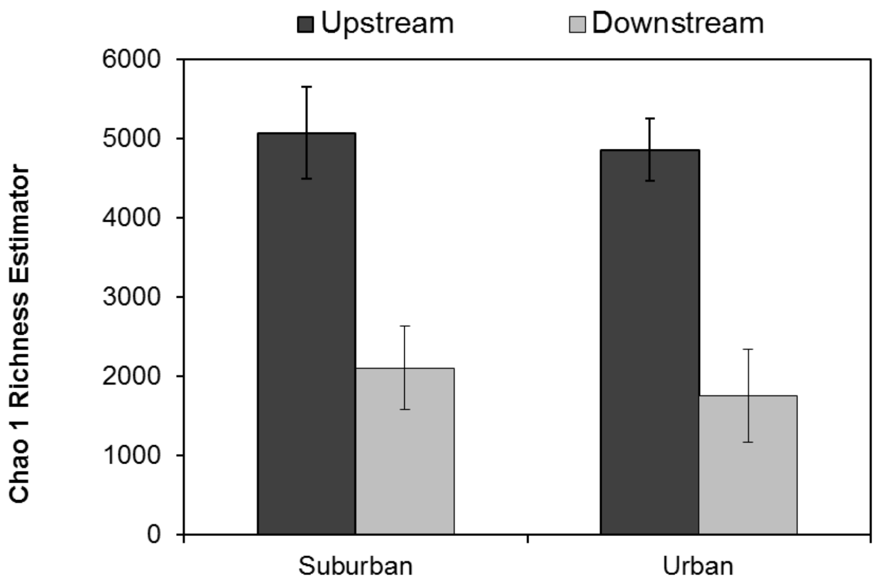
Drury et al., 2013, *Appl Environ Microbiol*



Shannon Diversity Index

(Based on Pyrosequencing Data)

- No Significant Habitat Effect
- Significant Effluent Effect
- No Interaction



Chao 1 Richness

(Based on Pyrosequencing Data)

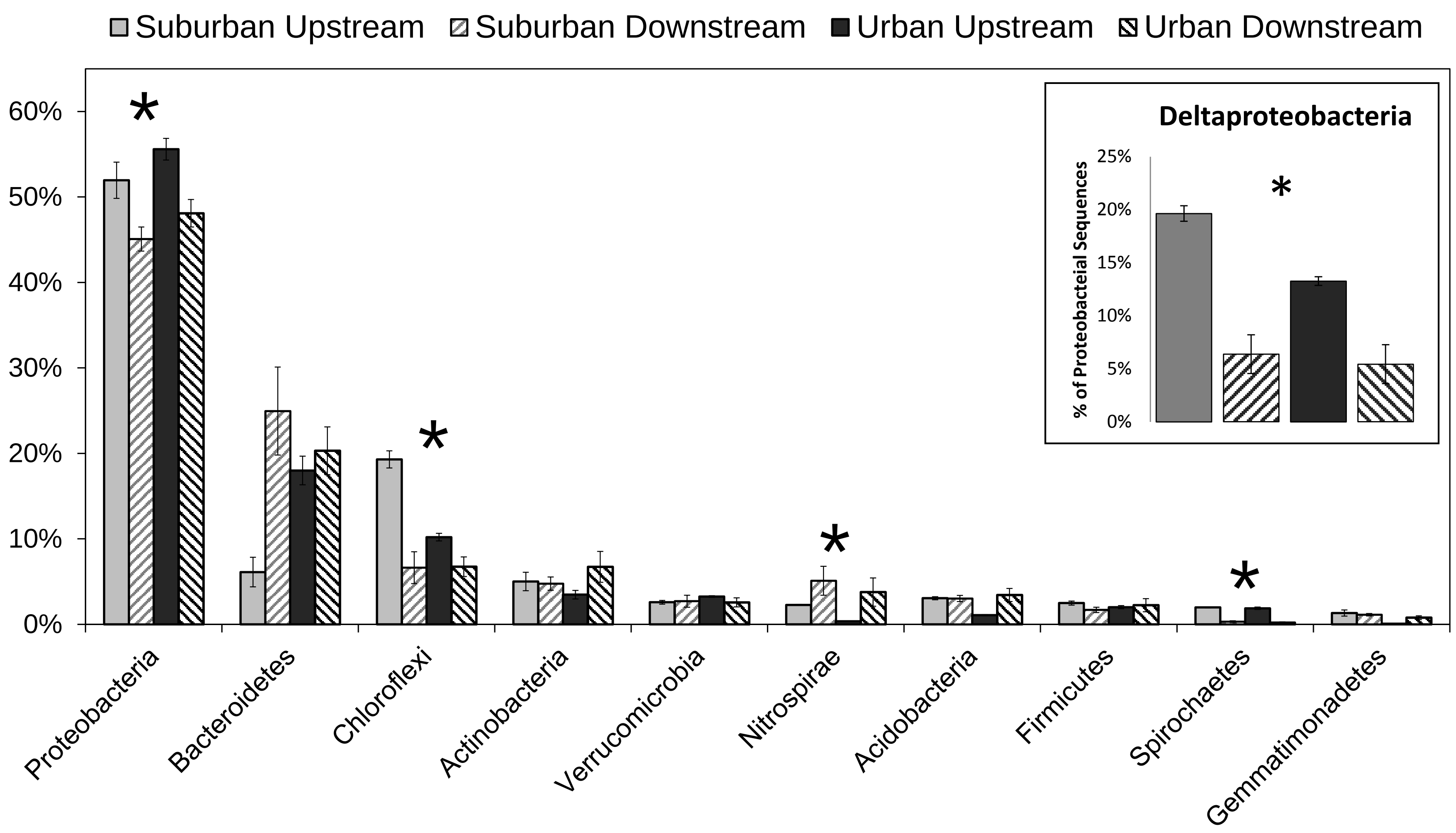
- No Significant Habitat Effect
- Significant Effluent Effect
- No Interaction

Mean (n=5) +/- Standard Error

Drury et al., 2013, *Appl Environ Microbiol*

Abundance of Dominant Bacterial Phyla

(Based on Pyrosequencing Data)



Drury et al., 2013, *Appl Environ Microbiol*

Bacterial OTUs That Varied with Effluent

(Based on Pyrosequencing Data)

Operational Taxonomic Units[c]	Relative Abundance (%)[a] All Upstream Sites	Relative Abundance (%)[a] All Downstream Sites	p value[b]	Contribution to variation (%)	Cumulative Contribution to variation (%)	Taxonomic Identification[d]
Otu4	0.16	4.35	0.043	2.51	2.51	*Sphingobacteriales*
Otu2	0.04	3.63	0.005	2.16	4.67	*Gallionellaceae*
Otu3	4.00	0.62	0.002	2.07	6.74	*Crenothrix*
Otu1	2.68	0.30	0.012	1.53	8.27	*Dechloromonas*
Otu42	0.38	2.73	0.002	1.41	9.69	*Verrucomicrobia*
Otu5	2.36	0.08	0.004	1.37	11.06	*Thiobacillus*
Otu8	2.97	0.71	$p<0.001$	1.36	12.41	*Desulfococcus*
Otu39	0.10	2.10	0.272	1.22	13.64	*Alteromonadaceae*
Otu16	1.67	0.11	$p<0.001$	0.94	14.58	*Proteobacteria* unclassified
Otu33	0.87	2.09	0.003	0.86	15.44	*Rhodobacter*
Otu12	1.43	0.10	0.017	0.85	16.29	*Comamonadaceae*
Otu6	0.45	1.26	0.399	0.78	17.07	*Deltaproteobacteria* unclassified
Otu10	0.00	1.26	0.106	0.76	17.83	*Oceanospirillales*
Otu7	0.03	1.23	0.239	0.73	18.55	*Methylophilaceae*
Otu9	0.02	1.17	0.321	0.70	19.25	*Flavobacteriaceae*
Otu15	0.00	1.06	0.331	0.63	19.89	*Sphingobacteriales*
Otu18	0.01	1.04	0.327	0.62	20.51	*Methylophilus*

[a] Each data point is mean (n=5).

[b] p value based on ANOVA comparison of all upstream and all downstream samples.

Drury et al., 2013, *Appl Environ Microbiol*

Points of Interest from Effluent Study

1. Biotic Homogenization (BH)

- A complex process whereby formerly distinct biota at different locations become more similar in composition over time.
- BH can be driven by natural processes, including geological disturbances.
- Human activities are currently driving BH on an unprecedented scale.
- Urbanization has been recognized as a significant driver of BH.
- Recent work suggests that BH is widespread in aquatic and terrestrial systems, in taxonomic groups ranging from plants to birds and fish.
- BH is a concern because of the potential for decreases in global biological diversity and ecosystem resilience.
- BH has become an important research agenda in population and community ecology.
- No studies have linked the concept of BH to bacterial communities.

Community Structure

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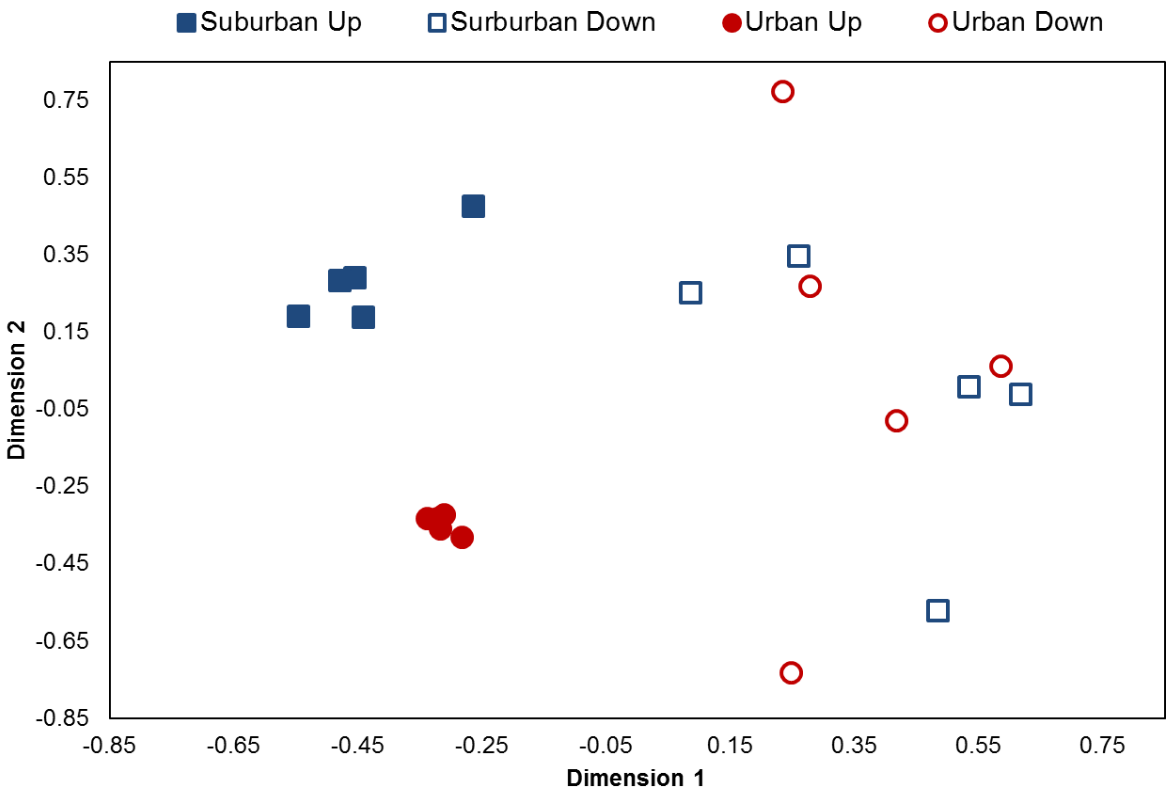

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Drury et al., 2013, *Appl Environ Microbiol*

Points of Interest from Effluent Study

2. Negative Impacts of WWTP Effluent

- At both the urban and suburban sites WWTP effluent resulted in increased inorganic nutrients (NO_3^- and PO_4^{3-})
- Previous work by others has demonstrated that increased concentrations of N and P associated with WWTP effluent:
  - Stimulates planktonic bacterial growth (Garnier et al., 1992; Goñi-Urriza et al., 1999)
  - Increases abundance and diversity of benthic bacteria (Wakelin et al., 2008)
- Yet in our study WWTP effluent resulted in
  - Decreased numbers of bacterial cells
  - Decreased bacterial diversity
  - Increased per cell respiration rates

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Does WWTP effluent contain compounds with antimicrobial properties?

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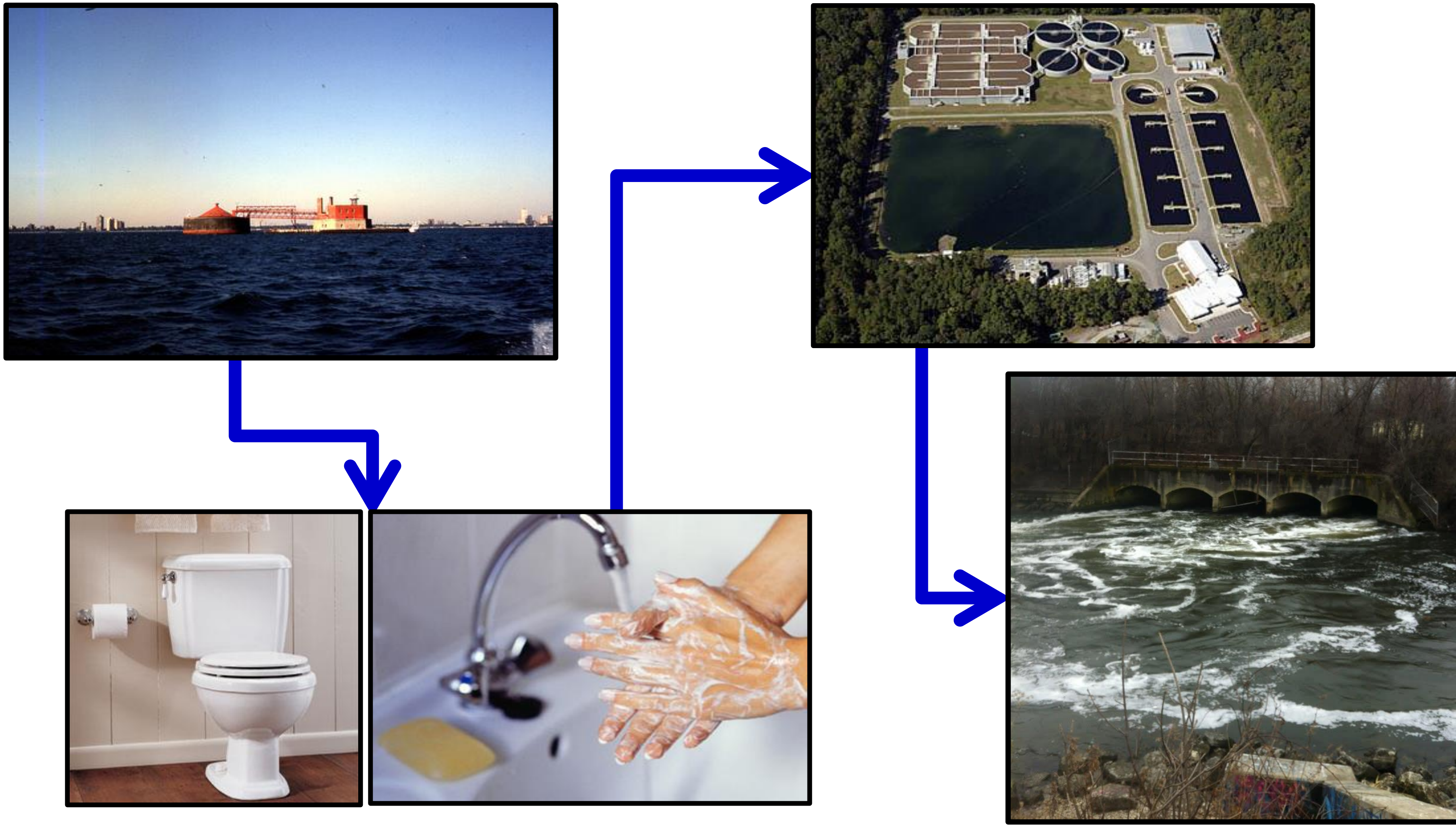

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  – Illinois Sustainable Technology Center
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  – Illinois Sustainable Technology Center
- Monte Wilcoxon
  – Illinois Sustainable Technology Center